Perspective

The PPARs: From Orphan Receptors to Drug Discovery[†]

Timothy M. Willson,* Peter J. Brown, Daniel D. Sternbach, and Brad R. Henke

Department of Medicinal Chemistry, Glaxo Wellcome Research & Development, Research Triangle Park, North Carolina 27709

Received November 8, 1999

Introduction

Dietary fat intake is an environmental factor that affects many aspects of human health.[1] Several lines of evidence suggest that common diseases of modern society are associated with high-fat Western diets combined with a sedentary lifestyle.[2] For example, diabetes, obesity, and cardiovascular disease are major causes of mortality and morbidity whose incidence tracks with the rate of industrialization in many countries.[3] Conversely, it has been established that caloric restriction with low-fat diets in rodents and primates leads to increased longevity and lower incidence of metabolic and cardiovascular disorders.[4] Interestingly not all dietary fat is bad; diets high in monounsaturated fatty acids (the Mediterranean diet) or polyunsaturated fatty acids (the Eskimo diet) appear to have cardioprotective effects.[5,6] The epidemiology of human metabolic diseases and animal feeding studies support the proposal that caloric intake plays an important role in the regulation of lipid metabolism, insulin sensitivity, glucose homeostasis, and atherosclerosis.[1,7] It is therefore not surprising that mammals have evolved with hormonal systems to regulate the physiological response to dietary intake of fatty acids.[8] A family of transcription factors known as the Peroxisome Proliferator-Activated Receptors (PPARs) plays a central role in regulating the storage and catabolism of dietary fats. The PPARs were cloned less than a decade ago as orphan members of the nuclear receptor gene family that includes the receptors for the steroid, retinoid, and thyroid hormones.[9,10] This review covers the rapid progress in the functional analysis of these orphan receptors, research which has led to a greater understanding of the importance of fatty acids as hormones and has established the PPARs as molecular targets for the development of drugs to treat human metabolic diseases.

Functional Domains and Bioassays

There are three PPAR subtypes, which are the products of distinct genes and are commonly designated PPARα [NR1C1], PPARγ [NR1C3], and PPARδ [NR1C2].[11] The PPARs possess a domain structure common to other members of the nuclear receptor gene family (Figure 1A). Sequence comparison of their DNA-binding domains (DBD) shows that they are highly conserved, while the ligand-binding domains (LBD) have a slightly lower level of conservation across the subtypes. Within the LBD certain conserved amino acids have been mapped to critical receptor functions involved in signal transduction. However, there is significant sequence variation in the residues that line the ligand-binding pocket,[12–14] which is reflected in the fact that each receptor subtype is pharmacologically distinct.[15] The N-terminal region of the receptor shows low sequence identity across the subtypes and is in general less well-characterized. Recent data suggests that the N-terminal domain is responsible for differences in the biological function of the subtypes.[16] In addition, phosphorylation of the PPAR N-terminal domain by mitogen-activated protein kinase can affect the transcriptional activity[17–21] and possibly ligand binding of the receptor.[22] The PPARs form heterodimers with another nuclear receptor, the 9-*cis*-retinoic acid receptor (RXR) [NR2B].[11] The PPAR/RXR heterodimers bind to DNA sequences containing direct repeats of the

[†] Dedicated to the memory of Gordon L. Hodgson, 1945–2000.
* Address correspondence to: Timothy M. Willson, Glaxo Wellcome Research & Development, NTH-M1421, Five Moore Dr., P.O. Box 13398, Research Triangle Park, NC 27709-3398. Tel: (919) 483-9875. Fax: (919) 315-0430. E-mail: tmw20653@glaxowellcome.com.

A

B

AGGTCA n AGGTCA
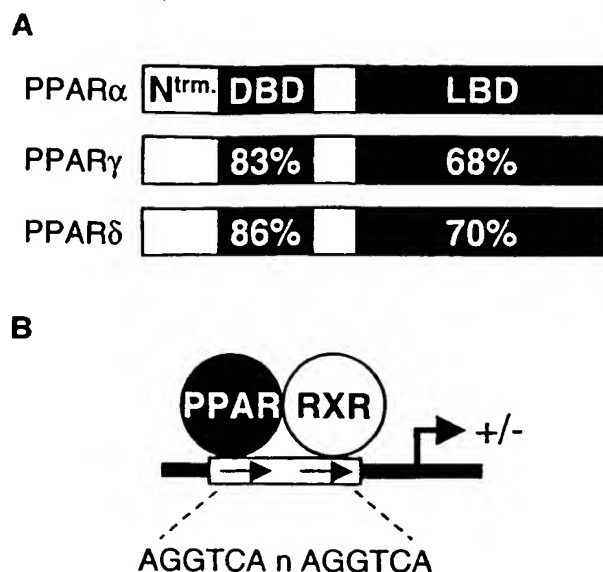
Figure 1. A. Functional domains of the PPARs: N^{trm}, N-terminus; DBD, DNA-binding domain; LBD, ligand-binding domain. Numbers represent the percent (%) identity between the human subtypes. B. PPAR/RXR heterodimer binds to a DR-1 response element in the regulatory regions of target genes. When an agonist ligand binds to the heterodimer, recruitment of coactivator proteins (not shown) leads to transcriptional activation.

hexanucleotide sequence AGGTCA separated by one nucleotide, known as DR-1 response elements (Figure 1B).[23] This sequence or minor variants have been characterized within the promoter regions of PPAR target genes such as acyl-CoA oxidase (*AOX*) and the adipocyte fatty acid-binding protein (*aP2*).[24]

In common with other members of the nuclear receptor gene family, the PPARs are ligand-activated transcription factors. The binding of agonist ligands to the receptor results in changes in the expression level of mRNAs encoded by PPAR target genes. This process is known as 'transactivation', and cell-based assays have been developed which monitor this functional activity. Transactivation assays use cells that have been transfected with a vector expressing the receptor as well as a second vector containing a DR-1 response element and a reporter gene, which encodes an enzyme such as chloramphenicol acetyltransferase, secreted placental alkaline phosphatase (SPAP), or firefly luciferase.[25] Activation of the receptor by agonist ligands leads to induction of reporter enzyme expression, which can be conveniently assayed using standard colorimetric or photometric plate readers. Either PPAR or RXR ligands can induce reporter expression, indicating that the PPAR/RXR heterodimer is the functional transcription factor within cells.[23] Although the standard transactivation assay is a powerful and widely used assay, the results can be complicated by the presence of other nuclear receptors within the host cells that bind to the same DR-1 response element as the PPAR/RXR heterodimer. Modified variants of the transactivation assay exploit the observation that the LBD and DBD are autonomous domains, such that they maintain their ligand- or DNA-binding properties when expressed as chimeric proteins with other nuclear receptors or transcription factors.[26] Of particular note is the PPAR-GAL4 transactivation assay, which uses chimeric receptors

Table 1. Activity of PPAR Agonists in Cell-Based Transactivation Assays[a]

compound	murine receptor EC50 (μM)			human receptor EC50 (μM)		
	PPARα	PPARγ	PPARδ	PPARα	PPARγ	PPARδ
Wy-14643	0.63	32	ia at 100	5.0	60	35
clofibrate[b]	50	~500	ia at 100	55	~500	ia at 100
fenofibrate[b]	18	250	ia at 100	30	300	ia at 100
bezafibrate	90	55	110	50	60	20
GW 9578	0.005	1.5	2.6	0.05	1.0	1.4
troglitazone	ia	0.78	ia	ia	0.55	ia
pioglitazone	ia	0.55	ia	ia	0.58	ia
rosiglitazone	ia	0.076	ia	ia	0.043	ia
KRP-297	10	0.14	7.2	0.85	0.083	9.1
JTT-501[b]	4.3	0.089	ia	1.9	0.083	ia
SB 213068	0.93	0.10	ia	0.74	0.066	ia
GI 262570	ia	0.00035	ia	0.45	0.00034	ia
GW 1929	ia	0.013	ia	ia	0.0062	ia
GW 7845	ia	0.0012	ia	3.5	0.00071	ia
GW 0207	ia	0.14	ia	ia	0.044	ia
L-796449	7.6	0.010	0.023	0.0041	0.0052	0.0079
L-165041	ia	10	3.8	10	5.5	0.53
GW 2433	0.27	1.5	0.41	0.17	2.5	0.19

[a] All data was generated using the PPAR-GAL4 transactivation assay using an SPAP reporter as described (ref 177); ± 20%, $n \geq 3$; ia = inactive at 10 μM or the concentration indicated. [b] Data is for the active metabolite.

where the PPAR LBD is fused to the DBD of the yeast transcription factor GAL4 and employs a reporter gene containing a GAL4 response element.[27] Since mammalian cells do not contain GAL4, only the transfected PPAR-GAL4 chimeric receptors can activate the reporter gene, effectively eliminating interference from endogenous nuclear receptors. In general, PPAR agonists show comparable potency and efficacy in assays using either the PPAR-GAL4 chimeras or the full-length receptors. The potency and selectivity of several classes of PPAR agonists in the PPAR-GAL4 assay are presented in Table 1.

Biochemical and genetic studies of the mechanism of transcription regulation have identified a series of accessory proteins that bind to nuclear receptors in a ligand-dependent manner.[28,29] These so-called 'coactivator' proteins promote the initiation of transcription and can be loosely categorized into three classes: proteins with histone acetylase activity that remodel chromatin structure (e.g. SRC1[30,31] and CBP/p300[32]), members of the DRIP/TRAP complex that interact with the basal transcriptional machinery (e.g. PBP/TRAP220[33,34]), and those proteins with poorly defined function (e.g. PGC1,[35] RIP140,[36,37] and ARA70[38]). Cell-free ligand sensing assays have been developed that exploit the ligand-dependent recruitment of coactivator proteins to the receptor. The coactivator-dependent receptor ligand assay (CARLA) detects the binding of a ^{35}S-labeled fragment of SRC1 to the PPAR LBD on an acrylamide gel.[39] Recently, the use of fluorescence resonance energy transfer has allowed the development of homogeneous CARLA assays.[40] These assays can be used as high-throughput cell-free screens for the identification of new PPAR ligands and for the characterization of coactivator recruitment to the receptor.

Several radioligands are now available for use in conventional competition binding assays: [3H]GW 2331 for PPARα;[41] [3H]rosiglitazone (BRL49653),[27] [3H]AD-5075,[42] and [^{125}I]SB-236636[43] for PPARγ; and [3H]GW 2433[44] and [3H]L-783483[45] for PPARδ. Each of these radioligands is reported to show specific binding to the

corresponding PPAR subtype. Initial PPAR binding assays used gel filtration to separate the bound radioligand from the free ligand.[27,41] However this technique is not ideal for high-throughput screening. The use of scintillation proximity assay (SPA) technology has greatly increased the throughput of PPAR competition binding assays.[46,47] SPA beads emit light when held in close proximity to a suitable radionuclide (e.g. ^3H or ^{125}I). If the receptor is attached to the SPA bead, binding of a radioligand to the PPAR LBD leads to a readily detectable signal. Displacement of the radioligand by a test compound leaves the free radioligand in solution, where it can no longer promote emission by the SPA bead. This technology removes the need to separate free radioligand from the bound ligand, greatly simplifying automated high-throughput screening. The homogeneous format of the SPA binding assay allows determination of equilibrium binding affinities and also permits the use of relatively low-affinity radioligands.[46]

The biology of the PPARs has been driven, in large part, by the availability of potent and selective ligands for the receptors.[48] Through the use of binding and functional assays, several groups have reported the identification and optimization of PPAR ligands for each of the three subtypes (Table 1). These chemical tools have been used in a "reverse endocrinology" approach to uncover the role of the PPARs in human physiology and disease processes.[49]
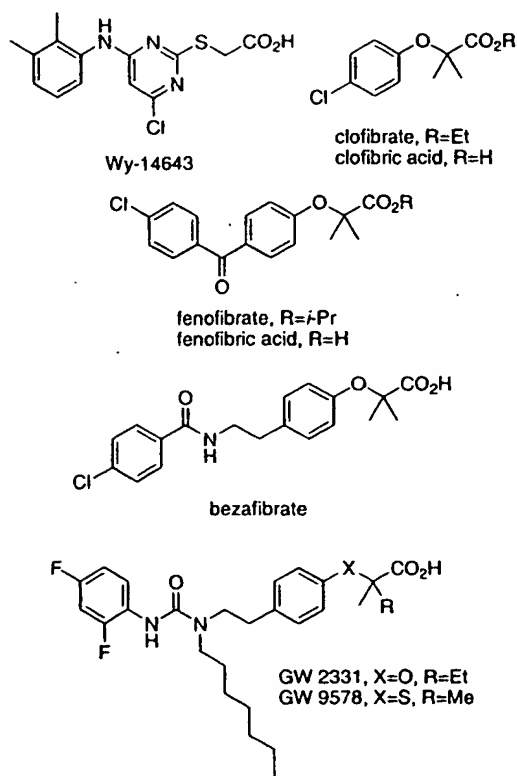
PPARα

PPARα was originally cloned from a mouse liver cDNA library[9] and has since been cloned from frogs,[50] rats,[26] guinea pigs,[51] and humans.[52,53] Although the DBDs are identical across a variety of species, the LBDs exhibit lower homology, which may reflect evolutionary adaptation to different dietary ligands. Comparison of human PPARα to the murine PPARα shows an 85% identity at the nucleotide level and 91% identity at the amino acid level. The human *PPARα* gene has been mapped to chromosome 22q12-q13.1 by somatic cell hybridization and linkage analysis.[52] Analysis of PPARα tissue distribution in rodents and humans revealed high levels of expression in metabolically active tissues, such as liver, heart, kidney, and muscle.[54,55] PPARα mRNA levels are under diurnal control of endogenous glucocorticoids in at least some of these tissues.[56,57]

PPARα is activated by a diverse range of compounds, such as fibrates and plasticizers, which cause the proliferation of peroxisomes and hepatomegaly in rodents.[9] This phenomenon has not been observed in nonrodent species, including humans, even though compounds that cause peroxisome proliferation activate both human and murine PPARα in cell-based transactivation assays.[58] Thus, although administration of peroxisome proliferators to rodents results in hepatomegaly and hepatocellular proliferation accompanied by suppression of apoptosis,[59] years of clinical experience with fibrates in humans has not led to evidence of peroxisome proliferation, as determined by morphological examination of liver biopsies,[60,61] or to an increased incidence of liver cancer.[62-64] The molecular basis of this species-specific response may be due to differences in the function of PPARα in rodents and humans. For example, there are differences in the hepatic expression

of PPARα across species. The wild-type PPARα is expressed in rodent livers at 10 times higher levels than in human livers.[65] In addition, a splice-variant of human PPARα, which represents 20−50% of the total PPARα mRNA in the liver, has been identified in which exon 6 is deleted.[65,66] The truncated human receptor lacks a LBD and was also unable to bind to a DR-1 response element. Although the truncated isoform inhibited the activity of wild-type receptor under certain conditions, immunolocalization showed that it was not normally present in the nucleus of cells.[66] A homologous truncated PPARα transcript could not be detected in rodent liver. Differences in the expression levels of PPARα and its isoforms may be only one factor affecting the species-specific response to peroxisome proliferators. It has been noted that the DR-1 response elements of key peroxisomal genes such as *AOX* are not conserved between rodents and humans.[67] Therefore, the physiological role of PPARα as a regulator of peroxisome function appears to be restricted to rodents, and the common designation of this receptor (and the other PPAR subtypes) does not reflect its biological function in humans.

Synthetic Ligands. The hypolipidemic fibrate drugs are an important class of PPARα ligands. Wy-14643 was identified as a micromolar activator of murine PPARα using a cell-based transactivation assay.[9] This drug as well as clofibrate, fenofibrate, and bezafibrate were developed as hypolipidemic agents through optimization of their lipid-lowering activity in rodents before the discovery of the PPARs. Table 1 shows the potencies of several fibrate drugs on the human and murine PPARs. Clofibric acid and fenofibric acid, the active metabolites of clofibrate and fenofibrate, are dual activators of PPARα and PPARγ, with ~10-fold selectivity for PPARα, while bezafibrate activates all three PPAR subtypes at comparable doses. All of these compounds require high micromolar concentrations to activate human PPARα, which may explain why high doses (300−1200 mg/day) are required for their clinical activity.[68] Attempts to identify more potent compounds led to the synthesis of a series of ureidofibrates that were active at lower doses in rodent models of hyperlipidemia.[69] One of these ureidofibrates, GW 2331, was shown to bind to both PPARα and PPARγ with nanomolar affinity.[41] Chemical libraries of ureidofibrates have been synthesized on solid-phase,[70] which has enabled this class of ligands to be rapidly optimized for PPAR activity on the human receptors.[44] The closely related ureidothioisobutyric acid GW 9578 has recently been reported as a potent and subtype-selective PPARα agonist with improved lipid-lowering activity compared to fenofibrate.[71]

Natural Ligands. Several groups have implicated fatty acids as natural ligands for PPARα. The Gustafsson laboratory was the first to demonstrate that a range of saturated and unsaturated fatty acids could activate PPARα.[26] A search for natural PPARα ligands in human serum identified palmitic acid, oleic acid, linoleic acid, and arachidonic acid as endogenous activators of rat PPARα.[72] Direct binding of fatty acids to PPARα has been demonstrated using competition binding assays[14,41] and cell-free ligand sensing assays.[39,73] Notably, PPARα is the only subtype that binds to a wide range of saturated fatty acids.[14] All of the fatty acids identified to date bind to PPARα with affinities in the micromolar

Wy-14643

clofibrate, R=Et
clofibric acid, R=H

fenofibrate, R=*i*-Pr
fenofibric acid, R=H

bezafibrate

GW 2331, X=O, R=Et
GW 9578, X=S, R=Me

range. While total fatty acid levels in serum reach these concentrations,[74] it is not known whether the free concentrations of fatty acids in cells are high enough to activate the receptor. This has prompted several investigators to search for high-affinity natural ligands among the known eicosanoid metabolites of polyunsaturated fatty acids. The lipoxygenase metabolite 8(*S*)-HETE was identified as a higher-affinity PPARα ligand,[39,41,73,75] although it is not found at sufficiently high concentrations in the correct tissues to be characterized as a natural ligand. Since no single high-affinity natural ligand has been identified, we and others have proposed that one physiological role of PPARα may be to sense the total flux of fatty acids in metabolically active tissues.[14,26,39,41,73]

palmitic acid

linoleic acid

8(S)-HETE
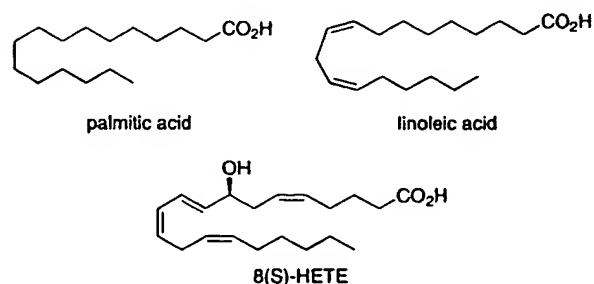
PPARα and Dyslipidemia. The discovery that the fibrates activate PPARα has triggered extensive research into the role of this receptor in mediating the lipid-lowering activity of these drugs. In humans, fibrates are effective at lowering serum triglycerides and raising HDL cholesterol (HDLc), primarily through increased clearance and decreased synthesis of triglyceride-rich VLDL.[76] Fibrates lower serum levels of apoC-III, a known inhibitor of VLDL clearance,[77] in both

humans[78] and rodents.[79] A PPAR response element has been identified in the proximal promoter of the *apoC-III* gene through which PPARα can inhibit its expression.[80] Response elements have also been identified in proximal promoters of other genes involved in lipid metabolism, such as *AOX*,[50] cytochrome P450 4A,[81] liver—fatty acid-binding protein (*L-FABP*),[82] and lipoprotein lipase (*LPL*).[83] In rodents apoA-I expression is down-regulated by fibrate treatment, leading to a reduction in HDLc. Since rodents carry most of their cholesterol in the HDL fraction, this is one pathway by which fibrates lower cholesterol levels in rodents. In humans, however, the reverse is true: fibrate treatment increases apoA-I expression leading to a rise in HDLc levels. This difference in gene regulation may be due to variations in the response element sequences in the rodent and human *apoA-I* genes.[84–86] The availability of a PPARα null mouse has aided the study of the role of the receptor in lipid homeostasis.[87] In contrast to wild-type mice, *PPARα*^{−/−} mice do not show decreases in serum lipid levels as a consequence of treatment with fibrates. Moreover, the null mice do not exhibit hepatomegaly, peroxisome proliferation, or induction of enzymes responsible for fatty acid oxidation when challenged with fibrates.[88]

The LDL cholesterol (LDLc)-lowering activity of the current fibrate drugs is weak compared to the statin class of drugs that inhibit HMG-CoA reductase.[89] At present, LDLc lowering is the primary endpoint by which lipid-lowering therapies are compared. Data are available showing a correlation of cardiac events with increasing LDLc levels, and cardiovascular outcome studies with statins have shown improvements in patient mortality and morbidity.[90] However, recent evidence has suggested that hypertriglyceridemia and low HDLc levels should also be considered when assessing a patient's cardiovascular risk factors.[91] Fibrates have been shown to slow the progression of atherosclerosis and reduce the number of coronary events in secondary prevention studies[92–94] and in patients with normal levels of LDLc.[95] Potent subtype-selective PPARα agonists, such as GW 9578, are more effective than the current fibrate drugs at lowering apoC-III levels in rodents.[71] Thus, drugs with potent activity on human PPARα may be useful adjuncts to current therapies for treatment of dyslipidemias in patients at risk of cardiovascular disease.

PPARα and Atherosclerosis. Factors other than dyslipidemia contribute to the development of coronary heart disease. Evidence is emerging that PPARα agonists may have direct effects in the arterial wall, which could contibute to the beneficial effects of these drugs in atherosclerosis prevention studies.[92–95] Atherosclerotic lesion formation requires recruitment of monocytes into the arterial wall through expression of adhesion molecules by activated endothelial cells.[96] Expression of the adhesion molecule VCAM-1 was down-regulated by PPARα agonists in human vascular endothelial cells.[97] This process was mediated in part by inhibition of NF-κB, a ubiquitous transcription factor that transduces the effects of many proatherogenic and inflammatory stimuli.[98] PPARα has also been shown to inhibit the actions of NF-κB in aged mice. Wy-14643 corrected the abnormal expression of genes under the control of

NF-κB in these mice[99] but was ineffective in aged PPARα^{-/-} mice.[100] Inflammatory processes have been implicated in disruption of the atherosclerotic plaque that leads to thrombolytic events.[101] PPARα agonists were shown to inhibit the IL-1-stimulated release of IL-6 and inflammatory prostaglandins in vascular smooth muscle cells.[102] Patients with coronary artery disease also responded favorably to fenofibrate treatment, showing reduced plasma levels of IL-6, fibrinogen, and C-reactive protein,[102] possibly through negative regulation of NF-κB and AP-1 by PPARα.[103] Additional evidence that PPARα can affect inflammatory processes was generated using the classical mouse ear-swelling test. Arachidonic acid-induced ear swelling lasted longer in PPARα^{-/-} mice compared to wild-type mice,[104] and it was hypothesized that PPARα may regulate genes involved in the catabolism of inflammatory eicosanoids. In support of this proposal the ear-swelling response to phorbol myristate acetate, which is not mediated by eicosanoids, was not affected by PPARα genotype.[104]

PPARα and Obesity/Diabetes. Obesity is a major risk factor for the development of diabetes, and fibrate treatment has been reported to reduce weight gain in rodents without effects on food intake.[105-108] Notably, PPARα^{-/-} mice show increased accumulation of body fat as they age.[109,110] These observations suggest that PPARα may affect body weight through regulation of fatty acid catabolism or energy expenditure.[111] Uncoupling proteins (UCP) 1−3 are mitochondrial membrane transporters that uncouple substrate oxidation from ATP synthesis, allowing conversion of fuel into heat.[112] Examination of the changes in UCP mRNA expression following bezafibrate treatment of rodents revealed increased levels of UCP1 in white adipose tissue (WAT) and increased levels of UCP3 in WAT and skeletal muscle.[113] In rat neonates Wy-14643 has been shown to induce the expression of *UCP3*,[114] a gene whose message levels are reduced in type 2 diabetics.[115] Three putative PPAR response elements have been identified in the *UCP3* promoter sequence.[116] Wy-14643 has been shown to transactivate the *UCP1* promoter in fetal rat brown adipocytes, although this did not result in increased UCP1 mRNA levels.[117] Under these same conditions, the PPARγ agonist rosiglitazone activated the *UCP1* promoter and increased UCP1 mRNA levels, but a combination of the PPARα and PPARγ agonists did not lead to increased UCP1 expression.[117] Interestingly, clofibrate and bezafibrate have been shown to improve glucose tolerance in type 2 diabetic patients.[118-120] However, the clinically used fibrates are only moderately selective for PPARα over PPARγ; thus it is not clear whether activation of the latter subtype is responsible for the observed effects. Confirmation of the therapeutic potential of PPARα agonists in obesity or diabetes will require clinical testing of drugs with greater subtype selectivity than the currently available fibrates.

PPARγ

PPARγ is the most extensively studied of the three PPAR subtypes to date. The receptor has been cloned from a number of species, including salmon,[121] mice,[15,122] hamsters,[123] frogs,[50] pigs,[124] rhesus monkeys,[125] and humans.[126-128] The human PPARγ protein is homologous to the murine PPARγ protein, with 95% identity at the amino acid level. In fact, the PPARγ protein shows a remarkable conservation across all the species from which it has been cloned, in contrast to that found thus far for PPARα and PPARδ. This high level of conservation may reflect the pivotal role that PPARγ plays as a regulator of glucose and lipid homeostasis, essential functions across all species. The human *PPARγ* gene has nine exons that extend over more than 100 kb of genomic DNA and has been mapped to human chromosome 3p25 by somatic cell hybridization and linkage analysis.[126,129]

Three mRNA isoforms of PPARγ have been detected in humans (termed PPARγ1, PPARγ2, and PPARγ3) which arise as products of different promoter usage.[130-132] PPARγ1 and PPARγ3 mRNAs code for the same protein, while PPARγ2 codes for a different protein containing 28 additional amino acids at the N-terminus. Analysis of the tissue distribution of the three human PPARγ isoforms revealed some differences. PPARγ1 had the broadest tissue expression, while the PPARγ2 and PPARγ3 isoforms had a more restricted distribution. Thus, PPARγ2 was expressed predominantly in adipose tissue,[130] and PPARγ3 was found only in adipose tissue, macrophages, and colon epithelium.[132,133] In addition to the aforementioned tissues, PPARγ1 mRNA was found in the heart, large and small intestines, colon, kidney, pancreas, spleen, and skeletal muscle. Cell culture experiments have demonstrated that PPARγ2 mRNA was induced during the early stages of adipocyte differentiation,[134-136] but PPARγ1 mRNA remained unchanged.[137] However, the functional significance of the multiple isoforms of PPARγ is currently unclear.[131,136,138]

PPARγ is a critical transcription factor in the regulation of adipocyte differentiation. Forced expression of PPARγ in fibroblasts in the presence of weak PPARγ activators resulted in differentiation of the cells to adipocytes.[135] Adipogenesis appears to require coordinated expression of the PPARγ and two other groups of transcription factors, C/EBP and ADD-1/SREBP-1. The detailed aspects of the interplay between PPARγ and these other transcription factors in adipocyte differentiation have been reviewed recently.[139,140] Adipocyte differentiation is accompanied by the induction of several genes involved in lipid homeostasis such as aP2,[136] PEPCK,[141] acyl-CoA synthetase (*ACS*),[142] and *LPL*.[83] All of these genes have been shown to contain PPAR response elements in their regulatory regions. PPARγ has also been shown to up-regulate the expression of the fatty acid transporters FATP-1 and CD36 in adipocytes.[143,144] These data demonstrate that PPARγ plays a pivotal role in the adipogenic signaling cascade and also suggest that the receptor can influence the production and cellular uptake of its own activators.

PPARγ has been shown to modulate a number of other genes involved in energy storage and utilization. Activation of PPARγ represses the expression of the *ob* gene, which codes for leptin.[145,146] PPARγ also appears to oppose the effects of another important adipocyte signaling factor, TNFα. Treatment of obese animals with PPARγ agonists reduces adipose tissue expression of TNFα.[147] PPARγ has been shown to up-regulate expression of the mitochondrial uncoupling proteins

UCP1, UCP2, and UCP3 in cells[148–151] and animals.[152,153] Recently, it was shown that activation of PPARγ in cultured adipocytes induced expression of c-Cbl associated protein, a potential signaling protein in insulin action.[154] The growing list of genes that are modulated by PPARγ continues to reinforce the notion that PPARγ is a key regulator of adipocyte function and systemic lipid homeostasis.

Synthetic Agonists. The first compounds reported as high-affinity PPARγ agonists were a class of antidiabetic agents known as thiazolidinediones (TZDs) or "glitazones".[27] The TZDs had been developed over a period of 15 years through empirical compound screening in rodent models of insulin resistance.[155] The molecular mechanism of action of the TZDs remained unknown until several reports in the mid-1990s suggested a possible connection between these agents and the PPARs. TZDs had been shown to induce gene expression in adipocytes and to enhance adipocyte differentiation in cell culture.[156,157] The TZD pioglitazone was also shown to increase expression of the *aP2* gene,[158] through a region of its promoter that had been independently identified by the Spiegelman group as a PPARγ binding site.[136] Through the use of binding and transactivation assays, the TZD rosiglitazone (BRL 49653) was identified as a high-affinity subtype-selective agonist for PPARγ.[27,159] In addition, the rank order of PPARγ potency of a number of TZDs has been shown to closely match their glucose-lowering activity in rodents.[42,160] These latter two pieces of data provide compelling evidence that PPARγ is the major receptor mediating the antidiabetic activity of the TZDs.

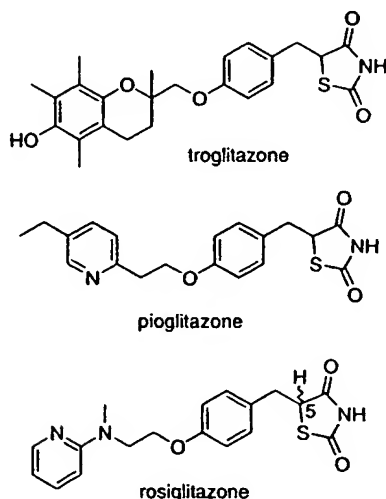troglitazone

pioglitazone

rosiglitazone

Table 1 lists the potency of those TZDs whose PPARγ activity has been reported. TZDs, in general, are selective for PPARγ over the PPARα and PPARδ subtypes,[27,160] although a TZD, KRP-297, with agonist activity at PPARα and PPARγ was recently disclosed.[161–163] The structurally related isoxazolidinedione JTT-501 has also been reported to activate PPARα at concentrations approximately 10-fold higher than those required for activation of PPARγ.[164] Its activity is likely to be mediated through a malonic amide metabolite that is generated by hydrolysis of the heterocyclic ring.[165] The TZD MCC-555 was reported to be a low-affinity but high-efficacy PPARγ agonist that showed potent an-

tidiabetic activity in rats.[166,167] Interestingly, MCC-555 appeared to function as a full or partial PPARγ agonist depending on the cell type and the response element used in the transactivation assay.[168] A series of antidiabetic Δ5-unsaturated-TZDs have also been reported, which were claimed to show little or no activity at PPARγ.[169,170] These observations raise the possibility that some TZDs mediate their antidiabetic activity through mechanisms other than PPARγ, although activation of PPARγ by metabolites of these drugs cannot be ruled out. An alternative explanation is that these compounds are able to produce tissue or promoter-specific modulation of PPARγ target genes, which was not detected by the standard PPARγ transactivation assays. Should this latter hypothesis prove valid, it will add a new dimension to the development of PPARγ ligands, similar to that stimulated by the selective estrogen receptor modulators in the osteoporosis field.[171]

JTT-501

JTT-501 metabolite
(malonic amide)

MCC-555

KRP-297
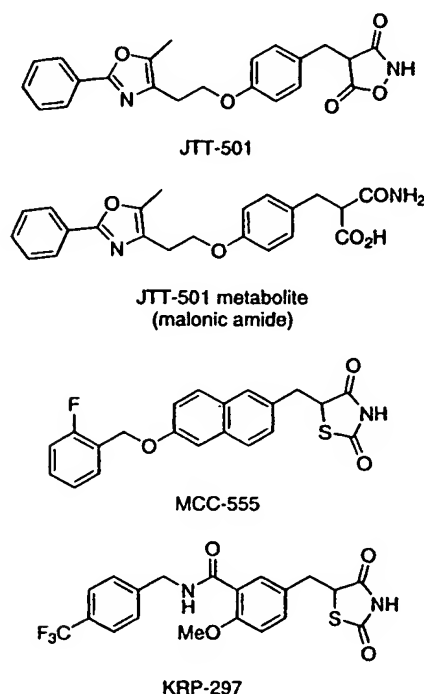
The TZDs contain a stereogenic center at C-5 of the heterocyclic headgroup but have been developed as racemates since they undergo racemization under physiological conditions.[172] Using a PPARγ binding assay it has been shown that only the (S)-enantiomers of the TZDs bind to the receptor with high affinity.[173] This surprising result suggests that only 50% of the drug substance in the currently approved TZDs binds to the target receptor, while 50% of the drug substance is inactive. To overcome this problem several groups have identified acyclic headgroups that are less prone to racemization. SB 213068 and its (S)-isomer, SB 236636, are representatives of a series of α-alkoxy-β-phenylpropanoic acids[174,175] that show agonist activity at PPARγ and PPARα (Table 1). Within this series the (S)-enantiomers were shown to have higher binding affinity for PPARγ and were more potent than their (R)-enantiomers in adipocyte differentiation assays.[43,176] Recently we reported a series of tyrosine-based PPARγ agonists exemplified by GI 262570, GW 1929, and GW

7845.[177-179] These compounds were the first antidiabetic drugs to be optimized based on their activity at human PPARγ. This series also contains some of the most potent PPARγ agonists reported to date, with a number of analogues having sub-nanomolar activity at human PPARγ (Table 1). In addition, these compounds showed >1000-fold selectivity for PPARγ over the PPARα and PPARδ subtypes in cell-based transactivation assays. GW 1929 demonstrated antihyperglycemic activity equal to troglitazone at >100-fold lower plasma concentrations in ZDF rats,[177,180] which parallels their differences in PPARγ binding and activation. Within this series the (S)-enantiomers, synthesized from naturally occurring L-tyrosine, were shown to have greater binding affinity and functional activity at PPARγ than the corresponding (R)-enantiomers.[177]

SB 213068 (R,S)
SB 236636 (S)

GI 262570

GW 1929

GW 7845
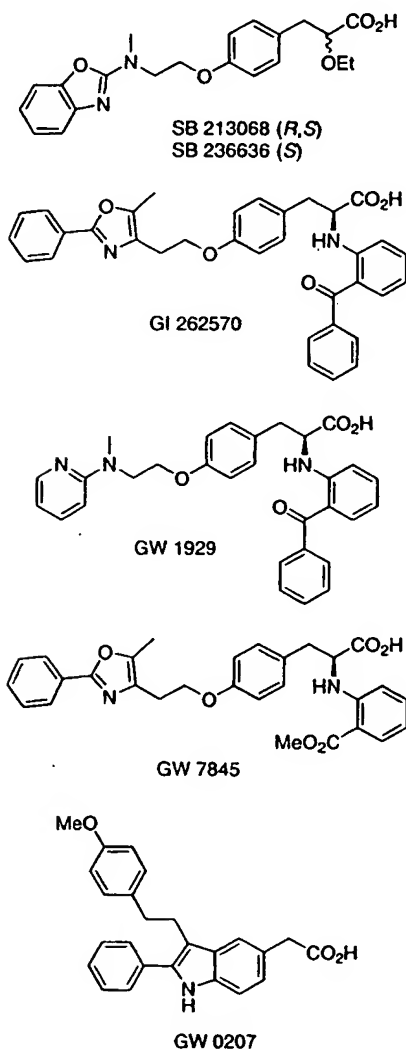
GW 0207

Other structurally diverse PPARγ agonists have been described in the literature. The 2,3-disubstituted indole-5-acetic acid derivative GW 0207 was a potent and selective PPARγ agonist.[181] Berger[45] has reported a series of phenylacetic acid derivatives, such as L-796449, which showed potent activity at PPARγ. Notably, L-796449 activated human PPARα and PPARδ at similar concentrations (Table 1), the first reported example of a potent agonist across all three receptor

subtypes. Several inhibitors of eicosanoid biosynthesis or receptor signaling pathways have also been reported to be weak agonists at PPARγ. The LTD4 receptor antagonist LY 171883 activated PPARγ in transactivation assays at micromolar concentrations.[15,159] The cyclooxygenase inhibitor indomethacin has been shown to bind and activate PPARγ at high micromolar concentrations.[182] Indomethacin was shown to promote adipocyte differentiation at concentrations similar to that required for activation of PPARγ.[183,184] Several other NSAIDs, including ibuprofen, fenoprofen, and flufenamic acid, were also shown to be weak PPARγ agonists.[182] It is important to note that interaction of these compounds with PPARγ occurs at higher concentrations than is required for inhibition of cyclooxygenase or LTD4 receptor antagonism.

Natural Ligands. The search for the natural PPARγ ligands has led to the discovery of a number of fatty acids and eicosanoid derivatives that bind and activate the receptor at micromolar concentrations. Unlike the PPARα subtype, PPARγ has a clear preference for polyunsaturated acids.[14] The essential fatty acids linoleic acid, linolenic acid, arachidonic acid, and eicosapentaenoic acid (EPA) have been shown to bind PPARγ at micromolar concentrations.[14,41] These values are within the range of concentrations of free fatty acids found in human serum;[74] however, as discussed earlier, it is unclear if these concentrations of fatty acids exist within cells. In fact, fatty acids are not particularly efficacious activators of PPARγ, and it has been demonstrated that intracellular conversion of fatty acids to eicosanoids, through enhanced expression of 15-lipoxygenase, resulted in increased PPARγ-mediated transactivation.[185] The 15-lipoxygenase metabolites of linoleic acid, 9-HODE and 13-HODE, have been shown to function as micromolar PPARγ agonists.[186] Therefore, metabolic conversion of polyunsaturated fatty acids within cells could provide an additional level of hormonal regulation of PPARγ. The J-series of prostaglandins derived from PGD2 have also been identified as PPARγ ligands.[75] The terminal metabolite 15-deoxy-Δ[12,14]-prostaglandin J2 (15d-PGJ2) activated PPARγ at low micromolar concentrations and also induced adipocyte differentiation.[159,187] This prostaglandin has become the most widely utilized naturally occurring PPARγ ligand. However, it is important to note that 15d-PGJ2 mediates some of its effects in cells through PPARγ-independent signaling pathways,[188-191] and caution must be exercised in attributing the biological effects elicited by 15d-PGJ2 solely to activation of PPARγ.

eicosapentaenoic acid

9-HODE

13-HODE

15-deoxy-Δ[12,14]-PGJ2
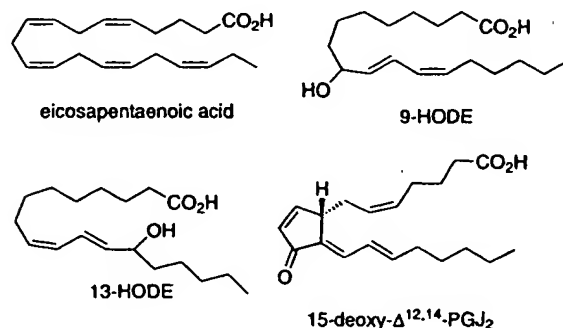
Partial Agonists and Antagonists. A novel PPARγ ligand GW 0072, which was identified from a diverse

combinatorial library, profiled as a partial agonist in transactivation assays and was an inhibitor of adipocyte differentiation in cell culture.[192] This compound had only 15–20% of the efficacy of rosiglitazone and was able to antagonize rosiglitazone in transactivation assays to the level of its own partial agonist activity with an IC_{50} = 150 nM. The low efficacy of GW 0072 was paralleled by its reduced ability to recruit coactivator proteins CBP and SRC1 to the receptor, which appears to be the result of a novel binding mode of the ligand within the PPARγ LBD (see below).[192] Although not a pure antagonist, GW 0072 may provide a valuable chemical tool for dissecting the pharmacology of PPARγ. Scientists at Merck have described a partial agonist, L-764406, which is an irreversible PPARγ ligand.[47] L-764406 binds covalently to Cys^{285} on helix 3 of the PPARγ LBD (Cys^{313} in PPARγ2). L-764406 displayed approximately 25% of the maximal activity obtained with TZDs, both in transactivation assays and in the induction of aP2 expression in 3T3-L1 preadipocyte cells. Antagonist activity of this compound was not reported. However, we have shown that an irreversible PPAR ligand, GW 9662, was able to antagonize the activation of PPARγ in macrophages.[185]

GW 0072

L-764406   GW 9662
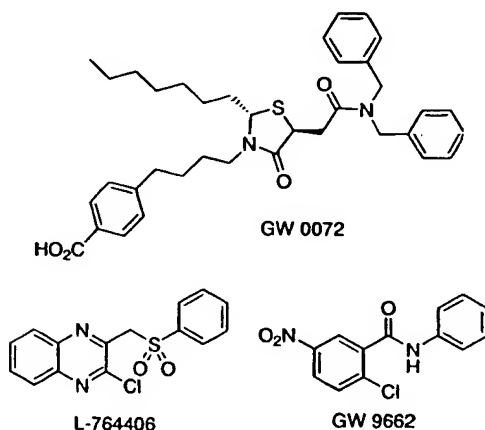
Protein Structure. The structure of the PPARγ LBD has been determined by X-ray crystallography. Structures have been solved in the absence of ligand (apo-PPARγ)[12,13] (Figure 2A) and bound to the TZD rosiglitazone together with an 88-amino acid fragment of the coactivator SRC1 (Figure 3).[12] The PPARγ LBD is a bundle of 13 α-helices and a small four-stranded β-sheet (Figure 2A), with an overall fold similar to other nuclear receptor structures from helix 3 to the C-terminus.[193] The crystal structure of the apo-PPARγ LBD revealed a large (~1300 Å³) Y-shaped ligand-binding site located within the bottom half of the LBD, extending from the C-terminal α-helix (known as AF-2) to the β-sheet between helices 3 and 6. The presence of an additional helix 2′ (Figure 2A), which is not seen in the steroid, retinoid, or thyroid receptor LBDs, may be responsible for the large volume of the PPARγ ligand-binding site.[12,13] Rosiglitazone binds in a U-shaped conformation, while occupying only 40% of the ligand-binding site. The TZD headgroup makes several specific hydrogen-bonding interactions with His^{449}, Tyr^{473}, His^{323}, Ser^{289}, and Gln^{286} (Figure 4A). Tyr^{473} lies on the C-terminal AF-2 helix, which is critical for transcriptional
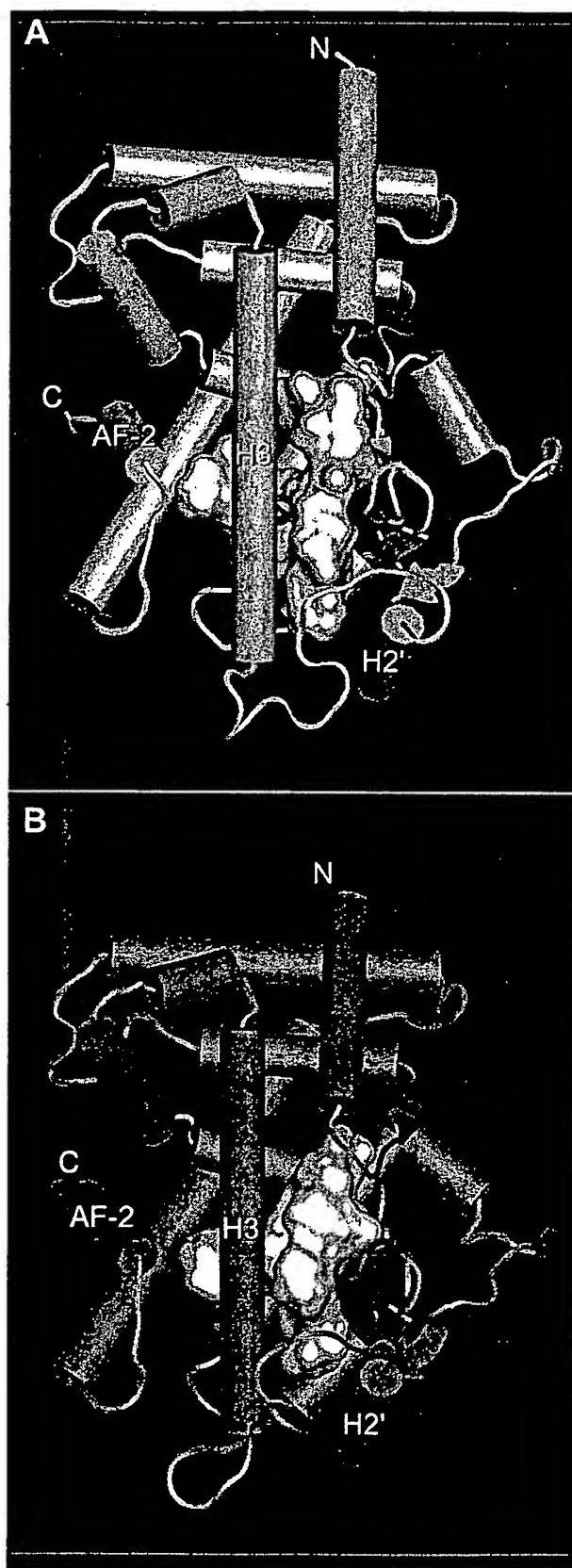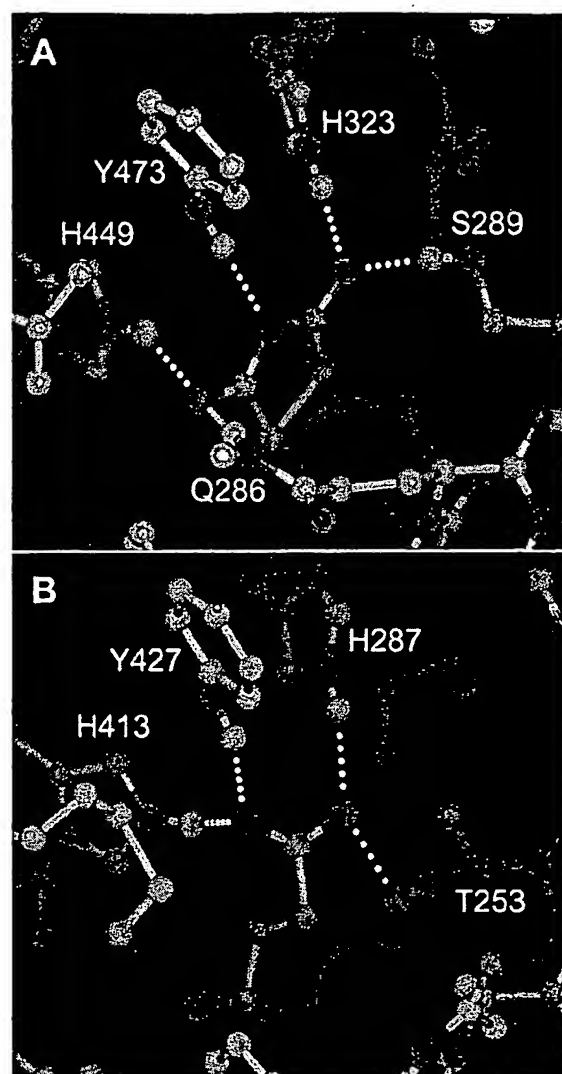

Figure 2. Crystal structures of the apo-PPAR LBDs. The α-helices are represented by tubes. Helix 2′, helix 3, and the AF-2 helix are designated as H2′, H3, and AF-2, respectively. The solvent-accessible ligand-binding site is indicated by the white shaded surface: A. PPARγ LBD; B. PPARδ LBD.
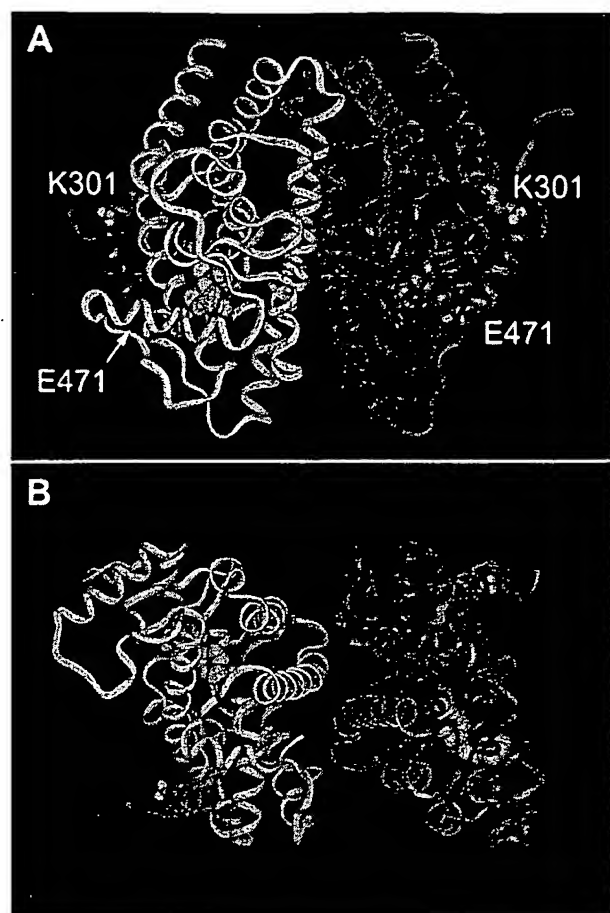
Figure 3. Crystal structure of the ternary complex of the PPARγ LBD with rosiglitazone and SRC1. Each PPARγ LBD of the homodimer is shown as a yellow worm. The side chains of the charge clamp residues Lys[301] and Glu[471] are colored orange. The SRC1 fragment containing the LxxLL motifs is shown in purple, but the linking peptide which showed poor electron density has been omitted. The TZD ligand is color coded by atom: A. side view of the complex, Lys[301] and Glu[471] are indicated; B. bottom view of the complex.

Figure 4. Conserved hydrogen-bonding interactions of PPAR agonists with the LBD. The key amino acids are indicated with their hydrogen bonds to the ligand drawn as white broken lines: A. TZD headgroup of rosiglitazone bound to PPARγ; B. carboxylic acid headgroup of EPA bound to PPARδ.

activation and provides the first example of a nuclear receptor ligand that interacts directly with a residue in this helix. Interestingly, in both the apo and ligand-bound structures of PPARγ, the AF-2 helix adopted a conformation similar to other ligand-bound nuclear receptors.[193] Although these structures do not provide clear evidence of a ligand-induced conformational change of the AF-2 helix, it is likely that interaction of the ligand with this helix locks the receptor in an activated state.

The ternary complex of PPARγ with rosiglitazone and SRC1 revealed the receptor crystallized as a homodimer bridged by a single molecule of the coactivator (Figure 3).[12] The homodimer is likely to use the same dimer interface as the physiologically relevant PPARγ/RXR heterodimer.[23] Each receptor in the dimer interacts with a short α-helix from the SRC1 fragment through a 'charge clamp' formed by Glu[471] on AF-2 and Lys[301] on helix 3 (Figure 3A). Each α-helix in the SRC1 fragment contains the peptide sequence LxxLL, where L = Leu and x = any amino acid. The charge clamp places the LxxLL motif into the optimal orientation to bury its Leu residues into a hydrophobic cleft on the surface of the

receptor (Figure 3B). Both LxxLL motifs make identical contacts with the receptor dimer, which may allow the multiple LxxLL motifs found in most coactivator proteins to mediate cooperative binding to nuclear receptor dimers. These structures revealed that binding of the PPAR ligand directly to the AF-2 helix stabilized the formation of the charge clamp on the surface of the receptor, which in turn is critical for recruitment of coactivator proteins to the receptor complex through their LxxLL motifs. Further support for this mechanism comes from the X-ray crystal structure of PPARγ bound to the partial agonist GW 0072.[192] In this structure (Figure 5), GW 0072 has a binding mode in which the ligand does not contact the AF-2 helix.[192] Although the backbone of the AF-2 helix was observed in the agonist-bound conformation, Tyr[473], His[323], and His[449] adopted side-chain conformations similar to those in the apo-PPARγ LBD crystal structure.[192] This suggests that in the GW 0072-bound receptor the charge clamp would not be stabilized through direct interactions with the ligand, which may account for the weak recruitment of

Figure 5. Partial agonist GW 0072 has a different binding mode to PPARγ compared to rosiglitazone. PPARγ LBD from the cocrystal with GW 0072 is shown as a yellow worm. GW 0072 is shown with its carbon atoms colored blue. Rosiglitazone was superimposed on the structure by alignment of the corresponding PPARγ LBD protein (not displayed). Rosiglitazone is shown with its carbon atoms colored green.

coactivator proteins.[192] Thus, it appears that the large binding pocket of PPARγ is not only capable of binding a number of structurally diverse ligands (Figure 5) but can also be exploited to identify ligands with altered binding characteristics and modified receptor pharmacology.

PPARγ and Diabetes. The most extensively studied therapeutic utility for PPARγ agonists has been in the treatment of type 2 diabetes.[194] TZD PPARγ agonists have been shown to enhance the sensitivity of target tissues to insulin and to reduce plasma glucose, lipid, and insulin levels in animal models of type 2 diabetes as well as in humans.[195-199] Troglitazone (Rezulin), pioglitazone (ACTOS), and rosiglitazone (Avandia) have been approved by the FDA and are currently marketed agents for the treatment of type 2 diabetes. These drugs represent an important advance in the treatment of this disease when used as monotherapy or in combination with existing therapies. Troglitazone has produced significant reductions in plasma glucose, HbA_{1c}, insulin, and C-peptide levels, either alone or in combination with sulfonylureas or metformin in type 2 diabetics.[200-202] In addition, troglitazone therapy has been shown to have beneficial effects on serum lipids,[203] blood pressure, and cardiac output.[204] However, a significant number of diabetics fail to respond to troglitazone therapy, and concerns have been expressed about the potential for weight gain[205,206] or increased adipogenesis in bone marrow[207] that is seen with TZDs in rodents.

A much more serious issue with troglitazone is hepatotoxicity. Troglitazone has been linked to a number of cases of liver failure[208] that has resulted in its

withdrawal for use as monotherapy in newly diagnosed type 2 diabetics in the United States and withdrawal of the drug from the market in the United Kingdom. It is presently unclear whether the observed hepatotoxicity is mediated by PPARγ or by some other mechanism unique to troglitazone. Neither rosiglitazone nor pioglitazone has displayed the increased incidence of hepatic adverse events seen with troglitazone, suggesting that hepatotoxicity may not be a class effect of PPARγ agonists. Troglitazone has also been shown to activate the human pregnane X receptor (PXR) [NR1I2][11] at the concentrations needed to activate PPARγ.[209] PXR is a recently isolated orphan member of the nuclear receptor gene family that was shown to be a key transcriptional regulator of hepatic cytochrome P450 3A4 (*CYP3A4*) gene expression.[210-213] Since CYP3A4 is responsible for the oxidative metabolism of around 60% of all clinically used drugs, activation of PXR may be the molecular mechanism underlying the observed drug–drug interactions of troglitazone.[214,215] Troglitazone is also a substrate for CYP3A4, leading to formation of a potentially reactive quinone metabolite.[216,217] Thus, the cross-reactivity of troglitazone on PXR may lead to increased hepatic levels of the quinone metabolite in some patients. In addition, other known activators of PXR cause hepatomegaly in rats, suggesting that PXR may regulate additional genes involved in liver metabolism.[210] PXR is remarkably divergent across species, and troglitazone does not activate the mouse or rat receptor.[209] This may explain why hepatotoxicity was not seen in the rodent safety studies of the drug. While it remains to be proven that activation of PXR by troglitazone is responsible for the hepatotoxicity in humans, screening of new PPARγ agonists for selectivity against PXR should lead to the development of diabetes drugs with reduced propensity for P450 enzyme induction and drug–drug interactions.[209]

RXR agonists are also known to activate the PPAR/RXR heterodimer and can show additive activity with PPAR agonists in cell-based transactivation assays.[23,218] Interestingly, targretin (LGD1069) and LG100268, selective RXR agonists that had been developed primarily as anticancer drugs, have shown glucose- and lipid-lowering activity in rodents.[218,219] These effects are likely to be mediated through activation of the PPARγ/RXR and PPARα/RXR heterodimers, although the activation of other RXR heterodimers cannot be ruled out. Notably, reports from human clinical trials indicate that these drugs may raise serum triglycerides[220] or lead to alterations in thyroid metabolism.[221,222] Thus, while it is still unclear that RXR agonists will be used as monotherapy for diabetes, they may be useful in combination therapy to increase the glucose- or lipid-lowering activity of various PPAR agonists.

The discovery that PPARγ is the molecular target of the antidiabetic TZDs has raised two apparent paradoxes. First, how can a receptor expressed mainly in adipose tissue improve insulin sensitivity and glucose utilization in skeletal muscle, the major insulin-sensing tissue? Several explanations have been proposed. Troglitazone has been shown to ameliorate insulin resistance and hyperglycemia in mice engineered to lack adipose tissue, suggesting that PPARγ action can occur by adipocyte-independent pathways.[223] Although PPARγ

levels are 10–100-fold higher in adipose than in muscle or liver, the receptor is expressed in these latter tissues.[131,224–226] Thus, stimulation of PPARγ in these non-adipose tissues could contribute to altered gene expression and a reduction in insulin resistance and improved glucose disposal. PPARγ may also exert direct effects on genes involved in glucose homeostasis, although surprisingly little is known about this topic. One potential target gene for PPARγ that is central to improving muscle glucose disposal is the insulin-dependent glucose transporter *GLUT4*. TZD activation of PPARγ has been shown to increase the expression of this gene in adipocytes,[227,228] but direct regulation of its expression in muscle has not been reported. UCP2, a mitochondrial uncoupling protein related to UCP1, has been shown to be expressed in a number of tissues including skeletal muscle and adipose tissue and appears to function as an important modulator of energy usage.[229] Expression of UCP2 was up-regulated by TZD activation of PPARγ in cell lines derived from skeletal muscle, WAT, and brown adipose tissue (BAT).[148,150] Finally, PPARγ agonists may regulate the storage or release adipocyte-derived signaling factors that affect insulin sensitivity in muscle. Fatty acids are key mediators of this process. It is well-established that increased fatty acid concentrations decrease glucose metabolism in muscle.[230] While PPARγ agonists induce LPL, FATP-1, and ACS in adipose tissue, their expression is apparently unchanged in muscle.[231] This may allow for an increase in fatty acid clearance into adipose tissue with concomitant decrease in uptake of fatty acids into muscle, potentially improving insulin sensitivity.[230] In support of this hypothesis TZD treatment is reported to reduce the triglyceride content of muscle in diabetic rats.[232] The mechanism of these effects may be related to the observation that PPARγ regulates expression of the fatty acid transporter CD36,[233] which was has been implicated in the control of insulin sensitivity in rats.[234] PPARγ-induced differentiation in WAT is also associated with a marked decrease in the levels of TNFα and leptin, two signaling molecules that are secreted by adipocytes.[235,236] Elevated levels of TNFα can cause insulin resistance,[237] and TZDs have been shown to block TNFα-induced insulin resistance in both cell culture[238] and animals.[239]

The second apparent paradox lies in the fact that obesity is a major risk factor in the development of type 2 diabetes. How then is a receptor that promotes adipogenesis still able to enhance insulin sensitivity? Evidence suggests that PPARγ-mediated differentiation of white adipocytes in rodents produces an increased number of small adipocytes while decreasing the number of large adipocytes.[235,236] These smaller adipose cells are usually more sensitive to insulin and would be expected to provide greater insulin-dependent glucose uptake. The smaller adipose cells should also have lower rates of lipolysis relative to larger adipose cells. Since high levels of free fatty acids have been linked to the induction of insulin resistance,[230,240,241] the decreased amount of circulating free fatty acid levels would be expected to have beneficial effects on insulin sensitivity. TZDs also induce the differentiation of BAT in rodents[152] and induce the expression of the mitochondrial uncoupling protein gene *UCP1* in human primary

adipocytes.[242] These effects may also contribute to increased energy consumption and decreased glucose and lipid levels.

Several polymorphisms have been identified in the human *PPARγ* gene. A rare Pro[115]Gln mutation in the PPARγ2 N-terminal domain, resulting in a constitutively active receptor, was identified in four obese German individuals.[243] This phenotype was consistent with PPARγ playing a role in the regulation of fat storage in humans. A more common silent C/T-polymorphism in exon 6 was detected in a sample of a northern French population that was associated with higher plasma leptin levels in obese subjects but not body mass index (BMI).[244] A relatively common Pro[12]Ala mutation in the PPARγ2-specific exon B has also been identified independently by two research groups.[245,246] Deeb found this mutation was associated with lower BMI, improved insulin sensitivity, and higher plasma HDLc concentrations.[246] The association with insulin sensitivity disappeared when corrected for BMI, indicating that the mutation primarily affects body weight. From a population of obese Finns, two women who were severely overweight were found to carry both the exon B and exon 6 polymorphisms.[247] However, other reports have found no association of the Pro[12]Ala mutation with BMI[248] and insulin sensitivity.[249] Subsequent reports[250–253] have failed to resolve this apparent conflict, underscoring the influence of environmental factors and the difficulty in determination of these phenotypes in humans.

In contrast to the human genetic studies, recent reports[254–256] of the phenotype of the PPARγ null mouse have shed additional light on the role of PPARγ in adipocyte function and insulin resistance. The PPARγ null mutation was embryonic lethal around day 10 of gestation due to a defect in placental development.[254,255] As expected, *PPARγ⁻/⁻* embryonic stem cells and fibroblasts failed to undergo differentiation to a mature adipocyte phenotype in cell culture.[255,256] Using a placental rescue technique Barak showed that PPARγ was not essential for further development of the mice.[254] A single mouse that was brought to full-term lacked adipose tissue and showed extreme metabolic defects, similar to the human condition of lipodystrophy.[257] These results confirmed the essential role of PPARγ in the formation of mature adipocytes and its important role in lipid and glucose homeostasis mediated through adipose tissue. Notably, the heterozygous *PPARγ⁺/⁻* mice develop normally, have a normal number and size of adipocytes, and show no metabolic defects. In an elegant experiment, Kubota reported that upon challenge with a high-fat diet, the *PPARγ⁺/⁻* mice were partially protected from weight gain and the development of insulin resistance when compared to their wild-type litter mates.[255] This surprising result was explained by the observation that the adipocytes of the *PPARγ⁺/⁻* mice were less hypertrophic, and the circulating leptin levels were 2-fold higher than in the wild-type mice. UCP expression was also 20% higher in the BAT of the *PPARγ⁺/⁻* mice. The differences were consistent with the decreased food intake and higher basal metabolic rate that was observed in the *PPARγ⁺/⁻* mice. These results raise the intriguing possibility of a dichotomy in PPARγ function with respect to insulin

sensitization.[255] On the one hand, PPARγ activation may promote adipocyte differentiation and the formation of small adipocytes leading to increased insulin sensitivity. On the other hand, PPARγ activation may cause hyperplasia of preexisting adipocytes and suppression of leptin production,[258] with a consequent decrease in insulin sensitivity. Thus, it is possible that what was once a thrifty gene, which evolved to aid the storage and release of fat during cycles of feast and famine, may now predispose modern humans to obesity and type 2 diabetes in our time of relative caloric excess.[259]

PPARγ and Dyslipidemia. It is well-established that PPARγ agonists decrease plasma levels of triglycerides, cholesterol, and nonesterified fatty acids in various animal models of dyslipidemia.[155] These lipid changes are also seen in humans, although the effects are less consistent. There appear to be some minor differences among the three marketed PPARγ agonists, although not enough clinical data are available yet on rosiglitazone and pioglitazone to afford a clear comparison. Troglitazone has shown a consistent decrease in plasma triglycerides (13–32%) and free fatty acids (up to 22%), while effects on cholesterol metabolism are variable, with some clinical trials reporting an increase in both HDLc (up to 16%) and LDLc (up to 13%) while others report no change in cholesterol levels.[202,260] In clinical trials, rosiglitazone has shown variable results on triglyceride lowering but appears to lower free fatty acids while raising both HDLc and LDLc.[261] Pioglitazone also lowers triglycerides and raises HDLc; however, LDLc does not appear to increase upon pioglitazone treatment, with levels being no different than placebo.[262] The origin of the observed differences in lipid effects among these TZDs remains unclear. On the basis of the current data, PPARγ agonists would appear to have some utility in lowering triglycerides and free fatty acids. However, no clinical trials with selective PPARγ agonists have been conducted on patient populations that are hyperlipidemic but not diabetic, so the pharmacological profile of these agents in a strictly dyslipidemic setting is unknown.

PPARγ and Hypertension. Hypertension is increasingly recognized as a complex metabolic and cardiovascular disorder. The pathogenesis of the disease involves improper regulation of blood pressure, insulin sensitivity, vascular function, and lipid metabolism.[263] TZDs have been shown to decrease blood pressure in a number of animal models, including Dahl S rats,[264] obese Zucker rats,[265,266] spontaneously hypertensive rats,[267] Watanabe rabbits,[268] and obese insulin-resistant rhesus monkeys.[269] Pioglitazone was reported to decrease blood pressure in the one-kidney, one-clip Sprague–Dawley rat, an animal model of hypertension not associated with insulin resistance.[270,271] This suggests that the ability of PPARγ agonists to prevent hypertension may be independent of their ability to improve insulin resistance. Troglitazone has been shown to reduce blood pressure in nondiabetic and diabetic humans[202] and improve overall cardiac output and stroke volume without an increase in cardiac mass.[204] The mechanism by which PPARγ agonists exert their antihypertensive effects is not well-understood. TZDs have been shown to inhibit growth factor-induced vascular smooth muscle

cell proliferation in cell culture[264,272] and in animals,[273,274] potentially through PPARγ-mediated inhibition of MAP kinase.[275,276] PPARγ agonists may also decrease blood pressure by affecting vascular contractility.[277] TZDs have been reported to modulate the production of several key factors involved in maintenance of vascular tone, including type-C natriuretic peptide,[278] endothelin,[278,279] and PAI-1.[280,281] Finally, TZDs have been shown to modulate currents in a number of ion channels. Troglitazone, pioglitazone, and rosiglitazone all suppress the voltage-gated (L-type) Ca²⁺ current in rat aortic myocytes.[282,283] However, the rank order of potency for Ca²⁺ channel inhibition does not correlate with potency for PPARγ activation, which suggests that the effect of these compounds on ion channels may be mediated through PPARγ-independent mechanisms.

PPARγ and Inflammation. The potential role of PPARγ in regulation of inflammatory processes was suggested by studies in adipose tissue, in which a general antagonism was seen between PPARγ and the proinflammatory cytokine TNFα. PPARγ agonists have been shown to reduce the expression level of TNFα in the adipose tissue of obese rats and to block the inhibitory effects of TNFα on insulin signaling[238] and TNFα-induced glycerol and free fatty acid release.[284] Monocytes and macrophages have a well-established involvement in inflammatory processes, including vascular wall inflammation and atherogenesis, through production of nitric oxide (NO) and inflammatory cytokines such as TNFα, IL-1, and IL-6. Chinetti[285] found that PPARγ expression was induced upon differentiation of human monocytes into macrophages. Activation of PPARγ by rosiglitazone or 15d-PGJ₂ resulted in induction of apoptosis due to interference with the NF-κB signaling pathway. Activation of PPARγ in macrophages has been shown to inhibit MMP-9 activity.[286] Treatment of activated macrophages with PPARγ agonists resulted in a change in cell morphology and suppression of NO production.[287] PPARγ agonists, including certain NSAIDs, block the production of inflammatory cytokines in human monocytes treated with phorbol myristate acetate.[288] However, it is important to point out that in both of these latter studies, the antiinflammatory activity seen with rosiglitazone occurred at concentrations considerably higher than the K_d value for binding to PPARγ or the concentration needed to elicit adipogenesis and insulin sensitization. Recent studies have demonstrated that activation of PPARγ by either 15d-PGJ₂ or rosiglitazone in colonic cell lines attenuated inflammatory cytokine gene expression through inhibition of NF-κB activation.[289] Furthermore, both troglitazone and rosiglitazone reduced colonic inflammation in an established murine model of colitis, suggesting that PPARγ agonists may have therapeutic potential in the treatment of inflammatory bowel disease.[289]

A recent report[233] also proposed that PPARγ might have proinflammatory or proatherogenic activity. PPARγ protein is abundantly expressed in foam cells of human atherosclerotic lesions.[133] Foam cells are cholesterol-laden macrophages that can accumulate below the arterial wall endothelium and contribute to atherosclerotic plaque formation. The conversion of macrophages to foam cells involves the internalization of oxidized

LDL (oxLDL) particles. This internalization does not occur through the LDL receptor but rather through lipid transporters such as CD36, SR-A, and others.[290] Treatment of HL60 monocytic leukemia cells with a combination of 15d-PGJ$_2$ and the RXR agonist LG100268 caused induction of macrophage markers and increased expression of CD36 and uptake of oxLDL.[233] The induction of CD36 suggests the possibility of an oxLDL–PPARγ–CD36 positive feedback loop.[233] However, it is important to point out that this induction of CD36 expression required the combination of a PPARγ agonist and an RXR agonist—treatment with a PPARγ agonist alone could not induce CD36. Thus it is also possible that RXR agonists contribute to foam cell formation through activation of other signaling pathways.

The results presented to date portray a somewhat conflicting story on the consequences of PPARγ activation in the context of inflammation and atherogenesis.[291] One difficulty in interpreting the results of the aforementioned experiments is that many investigators have employed the naturally occurring activator 15d-PGJ$_2$, which also has cellular activity independent of PPARγ. In addition most of the data has been generated in cell culture, where results are often difficult to extrapolate to human physiology. The current clinical data in humans suggests that troglitazone-mediated activation of PPARγ does not promote atherogenesis. Troglitazone has been shown to decrease serum PAI-1 levels in some insulin-resistant patient groups[292] and to reduce atherosclerosis by decreasing the intima and media thickness in carotid arteries of type 2 diabetics.[293] Thus, further studies are necessary to resolve these questions and explore the potential of PPARγ agonists in the treatment of inflammation and atheroscelerosis.

PPARγ and Cancer. Activation of PPARγ by TZDs has been shown to trigger cell cycle arrest in logarithmically growing NIH-3T3 fibroblasts and in malignantly transformed adipogenic HIB-1B cells.[294] The effects of PPARγ activation on differentiation and cell cycle regulation have prompted a number of investigations into the utility of PPARγ agonists for the treatment of cancerous tumors. Activation of PPARγ with pioglitazone in primary human liposarcoma cells has been shown to result in differentiation and withdrawal from the cell cycle.[295] Recently troglitazone was shown to induce terminal adipocytic differentiation in humans with advanced liposarcoma.[296] Since current chemotherapy regimens for inoperable liposarcomas are reported to give complete response rates in less than 10% of patients, PPARγ agonists have potential as chemotherapeutic agents for treatment of liposarcomas. PPARγ is also expressed in human primary and metastatic breast tumors.[297] Treatment of cultured breast cancer cells with troglitazone resulted in a reduction in growth rate and changes in cell morphology and gene expression representative of a more differentiated state. Interestingly, upon treatment with troglitazone for several days, the cells retained substantially reduced capacity for growth after cessation of drug treatment. Troglitazone has also been shown to inhibit tumor growth of MCF-7 cells in BNX triple-immunodeficient mice.[298] The tyrosine-based PPARγ agonist GW 7845 has been shown to significantly reduce tumor incidence, tumor size, and average tumor burden in the NMU-

induced rat model of mammary carcinoma.[299] Additive, but not synergistic, effects were seen with GW 7845 and a suboptimal dose of tamoxifen in this model. Consistent with these effects, expression of PPARγ is decreased in tumorigenic rodent mammary glands.[300]

PPARγ is expressed in human colon tumors and colon cancer cell lines.[301,302] Activation of PPARγ in these cell lines with PPARγ agonists caused inhibition of cell growth and cell cycle arrest, with morphologic changes consistent with colonic differentiation. Human colorectal cancer cells implanted in nude mice were shown to grow more slowly in mice treated with troglitazone, with a 50% reduction in tumor volume.[301] In addition, somatic mutations in PPARγ have been found in a subset of human primary colorectal carcinomas.[303] Each mutation led to a loss of receptor function when exposed to troglitazone or 15d-PGJ$_2$. These data suggest that activation of PPARγ might have a therapeutic benefit in the treatment of colorectal cancer. However, two groups have independently reported that PPARγ agonists increase the size and frequency of polyps in *APC*$^{Min/+}$ mice, an animal model genetically predisposed to intestinal neoplasia.[304,305] These mice are a model of the human genetic disease familial adenomatous polyposis coli (FAP), which is characterized by polyps and invasive neoplasia in the small and large intestines. In addition to FAP, the human *APC* gene is often mutated in sporadic colon cancer. It is not clear whether the increased polyp formation induced by activation of PPARγ in this murine model resulted in invasive carcinoma, as no clear examples were documented in either study. In addition, neither study found an increase in polyps in genetically nonsusceptible mice. Therefore, the potential for increased incidence of colon tumors in humans may be confined to those at risk of developing APC-negative cancers. Due to the discrepancy in these results,[301,303–305] however, further study of the role of PPARγ in colon carcinogenesis is needed.

Despite the apparent dichotomy in results with colon cells, these initial studies on the effects of PPARγ activation on cell cycle control have revealed the potential for using PPARγ as a therapeutic target in certain forms of cancer. Although extrapolation from cell culture and rodent studies to humans is complicated and often inaccurate, the antimitotic and differentiation-promoting abilities of PPARγ agonists warrant further human study. The fact that clinical use of troglitazone in diabetics has shown no overt toxicity in either colonic or breast tissue to date provides support for additional studies on the use of PPARγ agonists, either alone or in combination with more conventional chemotherapeutic agents, in the treatment of human cancers.

PPARδ

PPARδ has been cloned from a number of species and initially given a variety of names. The receptor was first reported as PPARβ in *Xenopus laevis*[50] and NUCI in humans.[306] Subsequently, the receptor was cloned from mice as PPARδ,[15] NUCI,[307] and FAAR.[308] Today, the generally agreed upon name for this receptor is PPARδ. The human and rodent receptors are about 90% identical in the LBD, while the frog receptor shows somewhat lower sequence identity (72%). Human PPARδ has been mapped to chromosome 6p21.1-p21.2. PPARδ mRNA is

ubiquitously expressed in adult rat tissues, but often at lower levels than either PPARα or PPARγ.[54] A study with human tissues showed that PPARδ was present in liver, intestine, kidney, abdominal adipose, and skeletal muscle, tissues that are all involved in aspects of lipid metabolism.[55]

Synthetic Ligands. Unlike the other subtypes, there are no known drugs that have been identified as working through PPARδ.[48] Thus, part of the challenge in determining the function of PPARδ has been the identification of potent and selective ligands for use as chemical tools.[49] It is important to note that all of the ligands published to date either have low affinity for PPARδ or lack selectivity over the other PPARs (Table 1). The Merck group, which originally cloned NUCI, has reported a series of synthetic ligands, such as L-631033, as weak activators of the receptor.[309] These compounds resemble fatty acids with a rigidifying ring in the middle of the chain, reminiscent of some eicosanoids. Recently this group has reported a more potent series of PPARδ agonists.[45] The established leukotriene antagonist[310] L-165041 was identified through random screening as an activator of human PPARδ. This compound shows 10-fold selectivity for human PPARδ over human PPARα and PPARγ but is only weakly active on the murine PPARδ with little selectivity over murine PPARγ (Table 1). The phenylacetic acid derivatives L-796449 and L-783483 were potent dual agonists of murine PPARδ and PPARγ but activated all three human PPAR subtypes. We have also identified a series of potent PPARδ ligands from a biased three-component library of ureidofibrates.[44] GW 2433 was a high-affinity ligand for human PPARδ and a dual activator of PPARδ and PPARα in cell-based transactivation assays (Table 1).
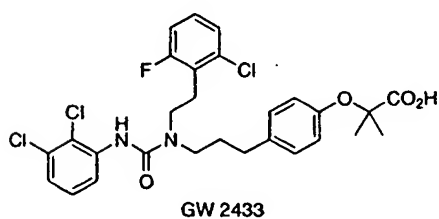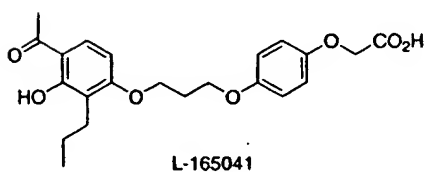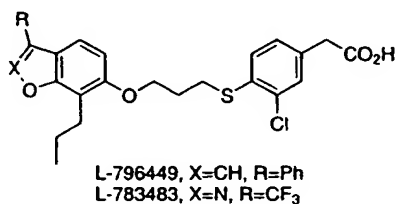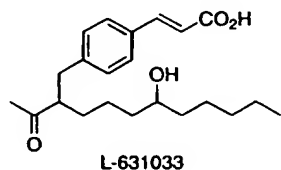
Natural Ligands. In common with the other subtypes, PPARδ is a receptor for naturally occurring fatty acids. A systematic study of the binding of fatty acids to PPARδ found that both saturated and unsaturated fatty acids bound to the receptor.[14] This binding profile was intermediate between that of PPARα and PPARγ. Among the polyunsaturated fatty acids, dihomo-γ-linolenic acid, arachidonic acid, and EPA bound with affinities in the low micromolar range.[14,73] In a search for natural ligands of PPARδ, methyl palmitate was isolated from acetone extracts of pancreatic tissue.[311] The fact that the free acid was not isolated as a PPARδ ligand is surprising, but the authors noted that the pancreas has high levels of enzymatic activity for conversion of fatty acids into their methyl esters. Another group[308] found that palmitic acid and the metabolically stable 2-bromopalmitic acid were activators of murine PPARδ. Several eicosanoids have been shown to activate PPARδ including PGA₁ and PGD₂.[75] The semisynthetic prostaglandin carbaprostacyclin was reported to be one of the most efficacious activators of PPARδ at micromolar concentrations.[73] Unfortunately, the naturally occurring prostacyclin (PGI₂) is too unstable to be assayed.

prostacyclin, X=O
carbaprostacyclin, X=CH₂
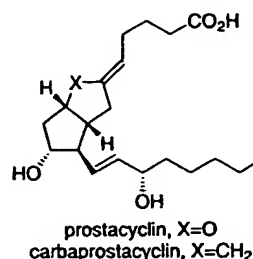
Protein Structure. The crystal structure of the apo-PPARδ LBD has been determined by molecular replacement, using the PPARγ crystal structure as a model (Figure 2B).[14] The overall structure of the PPARδ LBD was very similar to that of PPARγ including the general size of the ligand-binding pocket. However, differences in the residues lining the ligand-binding sites result in subtle changes to the shape of the pocket (Figure 2). A second structure was determined as a cocrystal with the natural ligand EPA (Figure 6).[14] Interestingly, EPA was observed bound to the receptor in two distinct conformations. The acid group and the first eight carbons adopted the same orientation in both conformations, while the tail (carbons 9–20) either pointed upward toward the upper lipophilic arm of the pocket or pointed downward into the third leg of the pocket. The ability of EPA to adopt multiple low-energy conformations within the receptor may be the molecular basis of the promiscuous binding of fatty acids to the PPARs and suggests that the need to sense multiple low-affinity hormones provided the evolutionary drive for the large ligand-binding site.[14] The carboxyl group of EPA was held in place by a network of hydrogen bonds from the side chains of His⁴¹³, Tyr⁴²⁷, His²⁸⁷, and Thr²⁵³ (Figure 4B). Tyr⁴²⁷ is part of the AF-2 helix, and the hydrogen bond formed with the ligand is likely to stabilize the conformation that facilitates coactivator recruitment. The same network of hydrogen bonds was observed in the ternary complex of PPARγ with rosiglitazone and SRC1 (Figure 4A).[12] The remarkable conservation of this

L-631033

L-796449, X=CH, R=Ph
L-783483, X=N, R=CF₃

L-165041

GW 2433
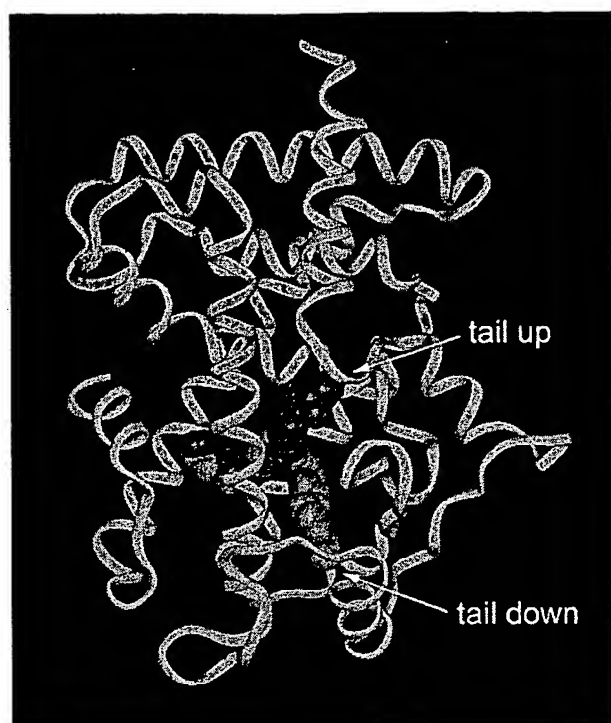
Figure 6. Crystal structure of EPA bound to the PPARδ LBD. The PPARδ LBD is shown as a blue worm. The two conformations of EPA observed in the crystal structure are shown in purple (tail up) and in green (tail down).

network of hydrogen bonds suggests that it must be crucial for ligand-mediated activation of these receptors. The cocrystal of PPARδ with GW 2433 revealed that this fibrate ligand also formed the same network hydrogen-bonding interactions with the AF-2 helix.[14] Interestingly, this molecule filled all the three legs of the Y-shaped pocket, which may explain why it bound with such high affinity to the receptor.

PPARδ and Dyslipidemia. It is likely that PPARδ is involved in lipid homeostasis because, like the other two subtypes, fatty acids and fatty acid metabolites activate the receptor. Amri showed that 3TC-C2 fibroblasts transfected with a PPARδ expression vector became responsive to fatty acids.[308] Specifically, two early markers of adipocyte differentiation, the adipocyte lipid-binding protein and the adipocyte fatty acid transporter (FAT/CD36), were induced. These effects may be due, in part, to PPARδ induction of PPARγ expression in the cells.[312] Pharmacological activation of PPARδ does not appear to have the same adipogenic potential as PPARγ.[313] The molecular basis for these differences was recently explored. Using a series of chimeric receptors created by domain swaps between PPARδ and PPARγ, Castillo reported that the N-terminal domain of PPARγ was largely responsible for adipogenic potential of the receptor.[16] Thus, a chimeric receptor containing the PPARδ LBD and DBD fused to the PPARγ N-terminal domain could induce adipogenesis when stimulated by 2-bromopalmitic acid. These results suggest that although the highly conserved LBD and DBD are essential for the function of the receptor, it is the poorly conserved N-terminus that dictates the adipogenic potential of the receptor.

The first proposed pharmacological role for PPARδ has been the regulation of cholesterol homeostasis.

Plasma cholesterol was raised in *db/db* mice treated with 30 mg/kg L-165041.[45] Although this compound shows only modest selectivity over PPARγ on the murine receptors (Table 1), the pharmacological effect was ascribed to activation of PPARδ since neither serum triglycerides nor glucose were lowered at this dose. Interestingly, in this same model a series of dual activators of PPARδ and PPARγ showed glucose-lowering activity, suggesting that activation of PPARδ did not inhibit insulin sensitization through PPARγ.[45]

PPARδ and Fertility. The role of PPARδ has been studied in blastocyte implantation and decidualization in mice.[314] PPARδ was the only subtype expressed in the uterus during the implantation period in mice. Expression was induced in the stroma surrounding the implanting blastocyst and became localized in the decidual zone post-implantation. This expression pattern was mirrored by the expression of prostacyclin synthase. Cyclooxygenase-2 (COX2) null mice show defects in implantation and decidualization. Since COX2 is involved in the synthesis of prostacyclin, it was hypothesized that $COX2^{-/-}$ mice may be deficient in endogenous activators of PPARδ.[314] In support of this hypothesis, PPARδ activators such as carbaprostacyclin or L-165041 in combination with 9-*cis*-retinoic acid were shown to restore implantation in the $COX2^{-/-}$ mice. Thus, prostacyclin or its metabolites may be regulators of embryo implantation though activation of PPARδ.

PPARδ and Cancer. A recent report has suggested that NSAIDs mediate their antitumorigenic activity in colorectal cancer in part through PPARδ.[315] High micromolar concentrations of the NSAIDs sulindac and indomethacin suppressed PPARδ activity in a transactivation assay. In colon cancer cells, sulindac-induced apoptosis was partially inhibited by adenoviral overexpression of PPARδ. The authors inferred that NSAIDs reduce the incidence of intestinal tumors by inhibiting PPARδ function and promoting apoptosis.[315] However, this proposal has yet to be tested using more potent PPARδ ligands.

Summary and Perspectives

It is nearly a decade since the first PPAR subtype was reported as an orphan member of the nuclear receptor gene family.[9] Few could have predicted in 1990 that the PPARs would emerge as therapeutic targets with widespread impact in the treatment of human metabolic diseases. The initial characterization of these orphan receptors focused on their roles as lipid-regulated transcription factors and the regulation of hepatic peroxisomal enzyme expression.[316] The role of these receptors in the induction of peroxisome proliferation is now understood to be species specific, with only rodents showing this response to activators of the PPARα subtype.[87] By contrast, all three PPARs appear to function as hormone receptors for dietary fatty acids and certain eicosanoids across multiple species and in many metabolically active tissues.[317]

A full appreciation of the therapeutic potential of these receptors required the identification of synthetic ligands that were drugs with formerly unknown mechanisms of action. The fibrate and glitazone drugs were developed by a succession of pharmaceutical companies over a period of 40 years using empirical medicinal

chemistry and rodent pharmacology. Although their cellular targets were unknown, these drugs had been successfully employed in the treatment of hypertriglyceridemia or type 2 diabetes in humans. The demonstration that PPARα and PPARγ were the receptors through which the fibrate[71] and glitazone[160] drugs mediate their biological activity has led to a renaissance in nuclear receptor research to develop drugs for diabetes and cardiovascular disease.[318,319] Knowledge of the molecular targets for the fibrates and glitazones has enabled medicinal chemists to synthesize a new generation of drugs that have been optimized for activity against the human PPARs. Several of these drugs are currently in clinical development. From our own laboratories, the selective PPARγ agonist GI 262570 is currently in phase III clinical trials for the treatment of type 2 diabetes.[320] Compounds with dual PPARγ and PPARα activity, which may combine the benefits of insulin sensitization and lipid lowering into a single drug, are also being investigated.[41,161,320] These dual agonists may be particularly effective in those diabetic patients that have the additional risk factor of high serum triglycerides.[321]

Even with the rapid progress that has occurred in characterization of these orphan receptors, several intriguing questions remain unanswered. What are the natural hormones and physiological functions of the PPARs? The accumulated evidence suggests that the PPARs are receptors for fatty acids and eicosanoids. However, it is still unclear how many of the currently identified naturally occurring ligands are true physiological hormones. To qualify for this designation, it needs to be demonstrated that a candidate ligand is found at sufficient concentrations in the correct tissues and cells and that its associated biology is physiologically relevant. For example, since PPARγ plays a critical role in regulation of lipid and glucose homeostasis through its role in adipocyte differentiation, we might expect that its natural hormone(s) would be associated with the epidemiology of type 2 diabetes.[5] What is the pharmacology of PPARδ ligands? While PPARδ is likely to be another fatty acid receptor,[14] its broad expression pattern gives few clues to its role in lipid metabolism or its other functions. This receptor will be a true test of the reverse endocrinology paradigm,[49] since PPARδ does not appear to be a receptor for any class of known drugs. The results of the study of PPARδ-selective ligands, especially in nonrodent animal models, are eagerly awaited. Do PPAR agonists have therapeutic utility beyond the metabolic disorders of diabetes and cardiovascular disease? Diets with a high content of polyunsaturated fatty acids have been claimed to show benefit in several chronic inflammatory diseases such as eczema, psoriasis, and arthritis.[322,323] In addition, the debate on the role of dietary saturated fat as an environmental factor in the development of cancer has raged for many years. The chemoprotective effects of polyunsaturated fatty acids in animal studies are supported by human epidemiological data on the incidence of cancer in Westernized nations; however these effects have yet to be confirmed in prospective human clinical trials.[324,325] The evaluation of synthetic PPAR agonists as drug therapies for nonmetabolic diseases will ultimately be required to answer this question.

The PPARs are now some of the most intensely studied members of the nuclear receptor gene family. Basic research on these receptors has increased our understanding of the molecular mechanism of hormone action, the role of fatty acids as hormones, and the role of adipose tissue as an endocrine organ. The promiscuity of the PPARs for their ligands and the availability of high-resolution X-ray structures has led to the development of new concepts about molecular recognition and the evolution of low-affinity hormone receptors. Throughout this research several apparent paradoxes in hormone function have come and gone, yet much remains to be explored. New technologies that enable transcript profiling through differential gene expression[326] will undoubtedly increase our knowledge of the mode of action of the PPARs through the identification of their key target genes. Thus, as we enter the 21st century, we may have the potential to develop PPAR drugs with increased efficacy and safety through understanding the molecular basis of their biological action. These drugs may be particularly useful in the growing population of prediabetic patients[2] that exhibit multiple risk factors for cardiovascular disease, such as impaired glucose tolerance and hypertriglyceridemia.[91,321] The success of these drugs will provide an acid test of the value of predictive molecular medicine over empirical drug discovery.

Biographies

Timothy M. Willson received his Ph.D. degree from the University of Southampton and completed his postdoctoral training at the University of Illinois at Urbana−Champaign. He joined the Glaxo Research Institute in 1990 and is currently Head of Nuclear Receptor Target Discovery at Glaxo Wellcome. His research focuses on the orphan members of the nuclear receptor gene family as targets for drug discovery.

Peter J. Brown received his Ph.D. degree from the University of Sheffield. Following postdoctoral training at Indiana University, he joined the Cardiovascular Chemistry Group at Bristol Myers. In 1989 he moved to the Glaxo Research Institute (now Glaxo Wellcome), where his current research involves the application of combinatorial chemistry to the discovery of orphan nuclear receptor ligands.

Daniel D. Sternbach received his Ph.D. degree from Brandeis University and completed his postdoctoral training at the ETH−Zurich and Harvard University. He joined the chemistry faculty at Duke University in 1979 and moved to the Glaxo Research Institute (now Glaxo Wellcome) in 1986. His research in medicinal chemistry has involved anticancer therapy and more recently metabolic diseases.

Brad R. Henke received his Ph.D. degree from the University of Illinois at Urbana−Champaign and completed his postdoctoral training at the University of California, Berkeley. He joined the Glaxo Research Institute in 1991 and is currently a Senior Research Investigator at Glaxo Wellcome. He led the chemistry team that identified the human PPARγ agonist GI 262570. His current research interests lie in the areas of metabolic and muscoskeletal diseases.

References

(1) Lichtenstein, A. H.; Kennedy, E.; Barrier, P.; Ernst, N. D.; Grundy, S. M.; Leveille, G. A.; Van Horne, L.; Williams, C. L.; Booth, S. L. Dietary fat consumption and health. *Nutr. Rev.* **1998**, *56*, S3−S28.

(2) Harris, M. I.; Flegal, K. M.; Cowie, C. C.; Eberhardt, M. S.; Goldstein, D. E.; Little, R. R.; Weidmeyer, H. M.; Byrd-Holt, D. D. Prevelence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The third National Health and Nutrition Examination Survey, 1988–1994. *Diabetes Care* **1998**, *21*, 518–524.

(3) Howard, B. V. Diet, insulin resistance, and atherosclerosis. *Int. Congr. Ser.* **1995**, *1100*, 446–450.

(4) Barzilai, N.; Gupta, G. Revisiting the role of fat mass in the life extension induced by caloric restriction. *J. Gerontol., Ser. A* **1999**, *54A*, B89–B96.

(5) Grundy, S. M. The optimal ratio of fat-to-carbohydrate in the diet. *Annu. Rev. Nutr.* **1999**, *19*, 325–341.

(6) Simopoulos, A. P. Evolutionary aspects of omega-3 fatty acids in the food supply. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1999**, *60*, 421–429.

(7) Lichtenstein, A. H. Dietary fat: a history. *Nutr. Rev.* **1999**, *57*, 11–14.

(8) Jump, D. B.; Clarke, S. D. Regulation of gene expression by dietary fat. *Annu. Rev. Nutr.* **1999**, *19*, 63–90.

(9) Issemann, I.; Green, S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **1990**, *347*, 645–650.

(10) Mangelsdorf, D. J.; Evans, R. M. The RXR heterodimers and orphan receptors. *Cell* **1995**, *83*, 841–850.

(11) A unified nomenclature system for the nuclear receptor super-family. *Cell* **1999**, *97*, 161–163.

(12) Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. *Nature* **1998**, *395*, 137–143.

(13) Uppenberg, J.; Svensson, C.; Jaki, M.; Bertilsson, G.; Jendeberg, L.; Berkenstam, A. Crystal structure of the ligand binding domain of the human nuclear receptor PPARγ. *J. Biol. Chem.* **1998**, *273*, 31108–31112.

(14) Xu, H. E.; Lambert, M. H.; Montana, V. G.; Parks, D. J.; Blanchard, S. G.; Brown, P. J.; Sternbach, D. D.; Lehmann, J. M.; Wisely, G. B.; Willson, T. M.; Kliewer, S. A.; Milburn, M. V. Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell* **1999**, *3*, 397–403.

(15) Kliewer, S. A.; Forman, B. M.; Blumberg, B.; Ong, E. S.; Borgmeyer, U.; Mangelsdorf, D. J.; Umesono, K.; Evans, R. M. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7355–7359.

(16) Castillo, G.; Brun, R. P.; Rosenfield, J. K.; Hauser, S.; Park, C. W.; Troy, A. E.; Wright, M. E.; Spiegelman, B. M. An adipogenic cofactor bound by the differentiation domain of PPARγ. *EMBO J.* **1999**, *18*, 3676–3687.

(17) Hu, E.; Kim, J. B.; Sarraf, P.; Spiegelman, B. M. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. *Science* **1996**, *274*, 2100–2103.

(18) Zhang, B.; Berger, J.; Zhou, G.; Elbrecht, A.; Biswas, S.; White-Carrington, S.; Szalkowski, D.; Moller, D. E. Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor γ. *J. Biol. Chem.* **1996**, *271*, 31771–31774.

(19) Adams, M.; Reginato, M. J.; Shao, D.; Lazar, M. A.; Chatterjee, V. K. Transcriptional activation by peroxisome proliferator-activated receptor γ is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. *J. Biol. Chem.* **1997**, *272*, 5128–5132.

(20) Camp, H. S.; Tafuri, S. R.; Leff, T. c-Jun N-terminal kinase phosphorylates peroxisome proliferator-activated receptor-γ1 and negatively regulates its transcriptional activity. *Endocrinology* **1999**, *140*, 392–397.

(21) Juge-Aubry, C. E.; Hammar, E.; Siegrist-Kaiser, C.; Pernin, A.; Takeshita, A.; Chin, W. W.; Burger, A. G.; Meier, C. A. Regulation of the transcriptional activity of the peroxisome proliferator-activated receptor α by phosphorylation of a ligand-independent trans-activating domain. *J. Biol. Chem.* **1999**, *274*, 10505–10510.

(22) Shao, D.; Rangwala, S. M.; Bailey, S. T.; Krakow, S. L.; Reginato, M. J.; Lazar, M. A. Interdomain communication regulating ligand binding by PPAR-γ. *Nature* **1998**, *396*, 377–380.

(23) Kliewer, S. A.; Umesono, K.; Noonan, D. J.; Heyman, R. A.; Evans, R. M. Convergence of 9-*cis* retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. *Nature* **1992**, *358*, 771–774.

(24) Wahli, W.; Braissant, O.; Desvergne, B. Peroxisome proliferator activated receptors: Transcriptional regulators of adipogenesis, lipid metabolism and more. *Chem. Biol.* **1995**, *2*, 261–266.

(25) McDonnell, D. P.; Vegeto, E.; Gleeson, M. A. G. Nuclear hormone receptors as targets for new drug discovery. *Bio/Technology* **1993**, *11*, 1256–1261.

(26) Göttlicher, M.; Widmark, E.; Li, Q.; Gustafsson, J. A. Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4653–4657.

(27) Lehmann, J. M.; Moore, L. B.; Smith-Oliver, T. A.; Wilkison, W. O.; Willson, T. M.; Kliewer, S. A. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). *J. Biol. Chem.* **1995**, *270*, 12953–12956.

(28) Freedman, L. P. Increasing the complexity of coactivation in nuclear receptor signaling. *Cell* **1999**, *97*, 5–8.

(29) Xu, L.; Glass, C. K.; Rosenfeld, M. G. Coactivator and corepressor complexes in nuclear receptor function. *Curr. Opin. Genet. Dev.* **1999**, *9*, 140–147.

(30) Zhu, Y.; Qi, C.; Calandra, C.; Rao, M. S.; Reddy, J. K. Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferator-activated receptor γ. *Gene Expression* **1996**, *6*, 185–195.

(31) Direnzo, J.; Soderstrom, M.; Kurokawa, R.; Ogliastro, M.-H.; Ricote, M.; Ingrey, S.; Horlein, A.; Rosenfeld, M. G.; Glass, C. K. Peroxisome proliferator-activated receptors and retinoic acid receptors differentially control the interactions of retinoid X receptor heterodimers with ligands, coactivators, and corepressors. *Mol. Cell. Biol.* **1997**, *17*, 2166–2176.

(32) Dowell, P.; Ishmael, J. E.; Avram, D.; Peterson, V. J.; Nevrivy, D. J.; Leid, M. p300 functions as a coactivator for the peroxisome proliferator-activated receptor α. *J. Biol. Chem.* **1997**, *272*, 33435–33443.

(33) Zhu, Y.; Qi, C.; Jain, S.; Rao, M. S.; Reddy, J. K. Isolation and characterization of PBP, a protein that interacts with peroxisome proliferator-activated receptor. *J. Biol. Chem.* **1997**, *272*, 25500–25506.

(34) Yuan, C.-X.; Ito, M.; Fondell, J. D.; Fu, Z.-Y.; Roeder, R. G. The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7939–7944.

(35) Puigserver, P.; Wu, Z.; Park, C. W.; Graves, R.; Wright, M.; Spiegelman, B. M. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **1998**, *92*, 829–839.

(36) Treuter, E.; Albrektsen, T.; Johansson, L.; Leers, J.; Gustafsson, J.-A. A regulatory role for RIP140 in nuclear receptor activation. *Mol. Endocrinol.* **1998**, *12*, 864–881.

(37) Miyata, K. S.; McCaw, S. E.; Meertens, L. M.; Patel, H. V.; Rachubinski, R. A.; Capone, J. P. Receptor-interacting protein 140 interacts with and inhibits transactivation by peroxisome proliferator-activated receptor α and liver-X-receptor α. *Mol. Cell. Endocrinol.* **1998**, *146*, 69–76.

(38) Heinlein, C. A.; Ting, H.-J.; Yeh, S.; Chang, C. Identification of ARA70 as a ligand-enhanced coactivator for the peroxisome proliferator-activated receptor γ. *J. Biol. Chem.* **1999**, *274*, 16147–16152.

(39) Krey, G.; Braissant, O.; L'Horset, F.; Kalkhoven, E.; Perroud, M.; Parker, M. G.; Wahli, W. Fatty acids eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **1997**, *11*, 779–791.

(40) Zhou, G.; Cummings, R.; Li, Y.; Mitra, S.; Wilkinson, H. A.; Elbrecht, A.; Hermes, J. D.; Schaeffer, J. M.; Smith, R. G.; Moller, D. E. Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer. *Mol. Endocrinol.* **1998**, *12*, 1594–1604.

(41) Kliewer, S. A.; Sundseth, S. S.; Jones, S. A.; Brown, P. J.; Wisely, G. B.; Koble, C.; Devchand, P.; Wahli, W.; Willson, T. M.; Lenhard, J. M.; Lehmann, J. M. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4318–4323.

(42) Berger, J.; Bailey, P.; Biswas, C.; Cullinan, C. A.; Doebber, T. W.; Hayes, N. S.; Saperstein, R.; Smith, R. G.; Leibowitz, M. D. Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-γ: binding and activation correlate with antidiabetic actions in *db/db* mice. *Endocrinology* **1996**, *137*, 4189–4195.

(43) Young, P. W.; Buckle, D. R.; Cantello, B. C. C.; Chapman, H.; Clapham, J. C.; Coyle, P. J.; Haigh, D.; Hindley, R. M.; Holder, J. C.; Kallender, H.; Latter, A. J.; Lawrie, K. W. M.; Mossakowska, D.; Murphy, G. J.; Cox, L. R.; Smith, S. A. Identification of high-affinity binding sites for the insulin sensitizer rosiglitazone (BRL-49653) in rodent and human adipocytes using a radioiodinated ligand for peroxisomal proliferator-activated receptor γ. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 751–759.

(44) Brown, P. J.; Smith-Oliver, T. A.; Charifson, P. S.; Tomkinson, N. C. O.; Fivush, A. M.; Sternbach, D. D.; Wade, L. E.; Orband-Miller, L.; Parks, D. J.; Blanchard, S. G.; Kliewer, S. A.; Lehmann, J. M.; Willson, T. M. Identification of peroxisome proliferator-activated receptor ligands from a biased chemical library. *Chem. Biol.* **1997**, *4*, 909–918.

(45) Berger, J.; Leibowitz, M. D.; Doebber, T. W.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C. A.; Hayes, N. S.; Li, Y.; Tanen, M.; Ventre, J.; Wu, M. S.; Berger, G. D.; Mosley, R.; Marquis, R.; Santini, C.; Sahoo, S. P.; Tolman, R. L.; Smith, R. G.; Moller, D. E. Novel peroxisome proliferator-activated receptor (PPAR) γ and PPARδ ligands produce distinct biological effects. *J. Biol. Chem.* 1999, *274*, 6718−6725.

(46) Nichols, J. S.; Parks, D. J.; Consler, T. G.; Blanchard, S. G. Development of a scintillation proximity assay for peroxisome proliferator-activated receptor γ ligand binding domain. *Anal. Biochem.* 1998, *257*, 112−119.

(47) Elbrecht, A.; Chen, Y.; Adams, A.; Berger, J.; Griffin, P.; Klatt, T.; Zhang, B.; Menke, J.; Zhou, G.; Smith, R. G.; Moller, D. E. L-764406 is a partial agonist of human peroxisome proliferator-activated receptor γ. The role of Cys^{313} in ligand binding. *J. Biol. Chem.* 1999, *274*, 7913−7922.

(48) Willson, T. M.; Wahli, W. Peroxisome proliferator-activated receptor agonists. *Curr. Opin. Chem. Biol.* 1997, *1*, 235−241.

(49) Kliewer, S. A.; Lehmann, J. M.; Willson, T. M. Orphan nuclear receptors: shifting endocrinology into reverse. *Science* 1999, *284*, 757−760.

(50) Dreyer, C.; Krey, G.; Keller, H.; Givel, F.; Helftenbein, G.; Wahli, W. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 1992, *68*, 879−887.

(51) Bell, A. R.; Savory, R.; Horley, N. J.; Choudhury, A. I.; Dickins, M.; Gray, T. J. B.; Salter, A. M.; Bell, D. R. Molecular basis of nonresponsiveness to peroxisome proliferators: the guinea-pig PPARα is functional and mediates peroxisome proliferator-induced hypolipidemia. *Biochem. J.* 1998, *332*, 689−693.

(52) Sher, T.; Yi, H. F.; McBride, O. W.; Gonzalez, F. J. cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry* 1993, *32*, 5598−5604.

(53) Mukherjee, R.; Jow, L.; Noonan, D.; McDonnell, D. P. Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J. Steroid Biochem. Mol. Biol.* 1994, *51*, 157−166.

(54) Braissant, O.; Foufelle, F.; Scotto, C.; Dauca, M.; Wahli, W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology* 1996, *137*, 354−366.

(55) Auboeuf, D.; Rieusset, J.; Fajas, L.; Vallier, P.; Frering, V.; Riou, J. P.; Staels, B.; Auwerx, J.; Laville, M.; Vidal, H. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-α in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes* 1997, *46*, 1319−1327.

(56) Lemberger, T.; Staels, B.; Saladin, R.; Desvergne, B.; Auwerx, J.; Wahli, W. Regulation of the peroxisome proliferator-activated receptor α gene by glucocorticoids. *J. Biol. Chem.* 1994, *269*, 24527−24530.

(57) Lemberger, T.; Saladin, R.; Vazquez, M.; Assimacopoulos, F.; Staels, B.; Desvergne, B.; Wahli, W.; Auwerx, J. Expression of the peroxisome proliferator-activated receptor α gene is stimulated by stress and follows a diurnal rhythm. *J. Biol. Chem.* 1996, *271*, 1764−1769.

(58) Cattley, R. C.; Deluca, J.; Elcombe, C.; Fenner-Crisp, P.; Lake, B. G.; Marsman, D. S.; Pastoor, T. A.; Popp, J. A.; Robinson, D. E.; Schwetz, B.; Tugwood, J.; Wahli, W. Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regul. Toxicol. Pharmacol.* 1998, *27*, 47−60.

(59) Roberts, R. A.; James, N. H.; Woodyatt, N. J.; Macdonald, N.; Tugwood, J. D. Evidence for the suppression of apoptosis by the peroxisome proliferator activated receptor α (PPARα). *Carcinogenesis* 1998, *19*, 43−48.

(60) Blumcke, S.; Schwartzkopf, W.; Lobeck, H.; Edmondson, N. A.; Prentice, D. E.; Blane, G. F. Influence of fenofibrate on cellular and subcellular liver structure in hyperlipidemic patients. *Atherosclerosis* 1983, *46*, 105−116.

(61) Hanefeld, M.; Kemmer, C.; Leonhardt, W.; Kunze, K. D.; Jaross, W.; Haller, H. Effects of p-chlorophenoxyisobutyric acid (CPIB) on the human liver. *Atherosclerosis* 1980, *36*, 159−172.

(62) Bentley, P.; Calder, I.; Elcombe, C.; Grasso, P.; Stringer, D.; Wiegand, H. J. Hepatic peroxisome proliferation in rodents and its significance for humans. *Food Chem. Toxicol.* 1993, *31*, 857−907.

(63) Ashby, J.; Brady, A.; Elcombe, C. R.; Elliott, B. M.; Ishmael, J.; Odum, J.; Tugwood, J. D.; Kettle, S.; Purchase, I. F. H. Mechanistically based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. Exp. Toxicol.* 1994, *13*, S1−S117.

(64) Heuvel, J. P. V. Peroxisome proliferator-activated receptors (PPARs) and carcinogenesis. *Toxicol. Sci.* 1999, *47*, 1−8.

(65) Palmer, C. N. A.; Hsu, M.-H.; Griffin, K. J.; Raucy, J. L.; Johnson, E. F. Peroxisome proliferator activated receptor-α expression in human liver. *Mol. Pharmacol.* 1998, *53*, 14−22.

(66) Gervois, P.; Torra, I. P.; Chinetti, G.; Grotzinger, T.; Dubois, G.; Fruchart, J.-C.; Fruchart-Najib, J.; Leitersdorf, E.; Staels, B. A truncated human peroxisome proliferator-activated receptor α splice variant with dominant negative activity. *Mol. Endocrinol.* 1999, *13*, 1535−1549.

(67) Lambe, K. G.; Woodyatt, N. J.; Macdonald, N.; Chevalier, S.; Roberts, R. A. Species differences in sequence and activity of the peroxisome proliferator response element (PPRE) within the acyl CoA oxidase gene promoter. *Toxicol. Lett.* 1999, *110*, 119−127.

(68) Gaw, A.; Packard, C. J.; Shepherd, J. Fibrates. *Handb. Exp. Pharmacol.* 1994, *109*, 325−348.

(69) Hawke, R. L.; Chapman, J. M.; Winegar, D. A.; Salisbury, J. A.; Welch, R. M.; Brown, A.; Franzmann, K. W.; Sigel, C. Potent hypocholesterolemic activity of novel ureido phenoxyisobutyrates correlates with their intrinsic fibrate potency and not with their ACAT inhibitory activity. *J. Lipid Res.* 1997, *38*, 1189−1203.

(70) Brown, P. J.; Hurley, K. P.; Stuart, L. W.; Willson, T. M. Generation of secondary alkylamines on solid-support by borane reduction. Application to the parallel synthesis of PPAR ligands. *Synthesis* 1997, 778−782.

(71) Brown, P. J.; Winegar, D. A.; Plunket, K. D.; Moore, L. B.; Lewis, M. C.; Wilson, J. G.; Sundseth, S. S.; Koble, C. S.; Wu, Z.; Chapman, J. M.; Lehmann, J. L.; Kliewer, S. A.; Willson, T. M. A ureido-thioisobutyric acid (GW9578) is a subtype-selective PPARα agonist with potent lipid-lowering activity. *J. Med. Chem.* 1999, *42*, 3785−3788.

(72) Banner, C. D.; Göttlicher, M.; Widmark, E.; Sjövall, J.; Rafter, J. J.; Gustafsson, J. A. A systematic analytical chemistry/cell assay approach to isolate activators of orphan nuclear receptors from biological extracts: characterization of peroxisome proliferator-activated receptor activators in plasma. *J. Lipid Res.* 1993, *34*, 1583−1591.

(73) Forman, B. M.; Chen, J.; Evans, R. M. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl. Acad. Sci. U.S.A.* 1997, *94*, 4312−4317.

(74) Jüngling, E. J.; Kammermeier, H. A one-vial method for routine extraction and quantification of free fatty acids in blood and tissue by HPLC. *Anal. Biochem.* 1988, *171*, 150−157.

(75) Yu, K.; Bayona, W.; Kallne, C. B.; Harding, H. P.; Ravera, C. P.; McMahon, G.; Brown, M.; Lazar, M. A. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* 1995, *270*, 23975−23983.

(76) Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J.-C. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 1998, *98*, 2088−2093.

(77) Shepherd, J. Lipoprotein metabolism: an overview. *Drugs* 1994, *47*, 1−10.

(78) Malmendier, C. L.; Lontie, J.-F.; Delcroix, C.; Dubois, D. Y.; Magot, T.; Der, L. Apolipoproteins C-II and C-III metabolism in hypertriglyceridemic patients. Effect of a drastic triglyceride reduction by combined diet restriction and fenofibrate administration. *Atherosclerosis* 1989, *77*, 139−149.

(79) Staels, B.; Ngoc, V.-D.; Kosykh, V. A.; Saladin, R.; Fruchart, J.-C.; Dallongeville, J.; Auwerx, J. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase: a potential mechanism for the hypolipidemic action of fibrates. *J. Clin. Invest.* 1995, *95*, 705−712.

(80) Hertz, R.; Bishara-Shieban, J.; Bar-Tana, J. Mode of action of peroxisome proliferators as hypolipidemic drugs. Suppression of apolipoprotein C-III. *J. Biol. Chem.* 1995, *270*, 13470−13475.

(81) Palmer, C. N. A.; Hsu, M. H.; Muerhoff, A. S.; Griffin, K. J.; Johnson, E. F. Interaction of the peroxisome proliferator-activated receptor α with the retinoid X receptor α unmasks a cryptic peroxisome proliferator response element that overlaps an ARP-1-binding site in the CYP4A6 promoter. *J. Biol. Chem.* 1994, *269*, 18083−18089.

(82) Issemann, I.; Prince, R.; Tugwood, J.; Green, S. A role for fatty acids and liver fatty acid binding protein in peroxisome proliferation? *Biochem. Soc. Trans.* 1992, *20*, 824−827.

(83) Schoonjans, K.; Peinado-Onsurbe, J.; Lefebvre, A.-M.; Heyman, R. A.; Briggs, M.; Deeb, S.; Staels, B.; Auwerx, J. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* 1996, *15*, 5336−5348.

(84) Berthou, L.; Duverger, N.; Emmanuel, F.; Langouet, S.; Auwerx, J.; Guillouzo, A.; Fruchart, J.-C.; Rubin, E.; Denefle, P.; Staels, B.; Branellec, D. Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J. Clin. Invest.* 1996, *97*, 2408−2416.

(85) Staels, B.; Auwerx, J. Regulation of apo A-I gene expression by fibrates. *Atherosclerosis* 1998, *137*, s19−s23.

(86) Vu-Dac, N.; Chopin-Delannoy, S.; Gervois, P.; Bonnelye, E.; Martin, G.; Fruchart, J.-C.; Laudet, V.; Staels, B. The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erbα mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. *J. Biol. Chem.* **1998**, *273*, 25713–25720.

(87) Lee, S. S.-T.; Pineau, T.; Drago, J.; Lee, E. J.; Owens, J. W.; Kroetz, D. L.; Fernandez-Salguero, P. M.; Westphal, H.; Gonzalez, F. J. Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **1995**, *15*, 3012–3022.

(88) Peters, J. M.; Hennuyer, N.; Staels, B.; Fruchart, J.-C.; Fievet, C.; Gonzalez, F. J.; Auwerx, J. Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor α-deficient mice. *J. Biol. Chem.* **1997**, *272*, 27307–27312.

(89) Gaw, A.; Shepherd, J. Fibric acid derivatives. *Lipoproteins Health Dis.* **1999**, 1145–1160.

(90) Ross, S. D.; Allen, I. E.; Connelly, J. E.; Korenblat, B. M.; Smith, M. E.; Bishop, D.; Luo, D. Clinical outcomes in statin treatment trials: a meta-analysis. *Arch. Intern. Med.* **1999**, *159*, 1793–1802.

(91) Grundy, S. M. Hypertriglyceridemia, atherogenic dyslipidemia, and the metabolic syndrome. *Am. J. Cardiol.* **1998**, *81*, 18B–25B.

(92) Oliver, M. F.; Heady, J. A.; Morris, J. N.; Cooper, J. WHO cooperative trial on the primary prevention of ischemic heart disease with clofibrate to lower serum cholesterol: final mortality follow-up. *Lancet* **1984**, *2*, 600–604.

(93) Ericsson, C. G.; Nilsson, J.; Grip, L.; Svane, B.; Hamsten, A. Effect of bezafibrate treatment over five years on coronary plaques causing 20% to 50% diameter narrowing (the bezafibrate coronary atherosclerosis intervention trial [BECAIT]). *Am. J. Cardiol.* **1997**, *80*, 1125–1129.

(94) Rutolo, G.; Ericsson, C.-G.; Tettamanti, C.; Karpe, F.; Grip, L.; Svane, B.; Nilsson, J.; De Faire, U.; Hamsten, A. Treatment effects on serum lipoprotein lipids, apolipoproteins and low-density lipoprotein particle size and relationships of lipoprotein variables to progression of coronary artery disease in the bezafibrate coronary atherosclerosis intervention trial (BECAIT). *J. Am. Coll. Cardiol.* **1998**, *32*, 1648–1656.

(95) Rubins, H. B.; Robins, S. J.; Collins, D.; Fye, C. L.; Anderson, J. W.; Elam, M. B.; Faas, F. H.; Linares, E.; Schaefer, E. J.; Schectman, G.; Wilt, T. J.; Wittes, J. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. *N. Engl. J. Med.* **1999**, *341*, 410–418.

(96) Libby, P.; Hansson, G. K.; Pober, J. S. Atherogenesis and inflammation. *Mol. Basis Cardiovasc. Dis.* **1999**, 349–366.

(97) Marx, N.; Sukhova, G. K.; Collins, T.; Libby, P.; Plutzky, J. PPARα activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. *Circulation* **1999**, *99*, 3125–3131.

(98) Thurberg, B. L.; Collins, T. The nuclear factor-κB/inhibitor of κB autoregulatory system and atherosclerosis. *Curr. Opin. Lipidol.* **1998**, *9*, 387–396.

(99) Spencer, N. F. L.; Poynter, M. E.; Im, S.-Y.; Daynes, R. A. Constitutive activation of NF-κB in an animal model of aging. *Int. Immunol.* **1997**, *9*, 1581–1588.

(100) Poynter, M. E.; Daynes, R. A. Peroxisome proliferator-activated receptor α activation modulates cellular redox status, represses nuclear factor-κB signaling, and reduces inflammatory cytokine production in aging. *J. Biol. Chem.* **1998**, *273*, 32833–32841.

(101) Kinlay, S.; Selwyn, A. P.; Libby, P.; Ganz, P. Inflammation, the endothelium, and the acute coronary syndromes. *J. Cardiovasc. Pharmacol.* **1998**, *32*, S62–S66.

(102) Staels, B.; Koenig, W.; Habib, A.; Merval, R.; Lebret, M.; Torra, I. P.; Delerive, P.; Fadel, A.; Chinetti, G.; Fruchart, J.-C.; Najib, J.; Maclouf, J.; Tedgui, A. Activation of human aortic smooth-muscle cells is inhibited by PPARα but not by PPARγ activators. *Nature* **1998**, *393*, 790–793.

(103) Delerive, P.; Bosscher, K. D.; Besnard, S.; Berghe, W. V.; Peters, J. M.; Gonzalez, F. J.; Fruchart, J.-C.; Tedgui, A.; Haegemann, G.; Staels, B. Peroxisome proliferator-activated receptor α negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1. *J. Biol. Chem.* **1999**, *45*, 32048–32054.

(104) Devchand, P. R.; Keller, H.; Peters, J. M.; Vazquez, M.; Gonzalez, F. J.; Wahli, W. The PPARα-leukotriene B₄ pathway to inflammation control. *Nature* **1996**, *384*, 39–43.

(105) Alegret, M.; Ferrando, R.; Vazquez, M.; Adzet, T.; Merlos, M. C.; Laguna, J. C. Relationship between plasma lipids and palmitoyl-CoA hydrolase and synthetase activities with peroxisomal proliferation in rats treated with fibrates. *Br. J. Pharmacol.* **1994**, *112*, 551–556.

(106) Alegret, M.; Cerqueda, E.; Ferrando, R.; Vazquez, M.; Sanchez, R. M.; Adzet, T.; Merlos, M.; Laguna, J. C. Selective modification of rat hepatic microsomal fatty acid chain elongation and desaturation by fibrates: relationship with peroxisome proliferation. *Br. J. Pharmacol.* **1995**, *114*, 1351–1358.

(107) Vazquez, M.; Munoz, S.; Alegret, M.; Adzet, T.; Merlos, M.; Laguna, J. C. Differential effects of fibrates on the acyl composition of microsomal phospholipids in rats. *Br. J. Pharmacol.* **1995**, *116*, 2067–2075.

(108) Vazquez, M.; Merlos, M.; Adzet, T.; Laguna Juan, C. Decreased susceptibility to copper-induced oxidation of rat-lipoproteins after fibrate treatment: influence of fatty acid composition. *Br. J. Pharmacol.* **1996**, *117*, 1155–1162.

(109) Gonzalez, F. J. Recent update on the PPARα-null mouse. *Biochimie* **1997**, *79*, 139–144.

(110) Costet, P.; Legendre, C.; More, J.; Edgar, A.; Galtier, P.; Pineau, T. Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* **1998**, *273*, 29577–29585.

(111) Torra, I. P.; Gervois, P.; Staels, B. Peroxisome proliferator-activated receptor α in metabolic disease, inflammation, atherosclerosis and aging. *Curr. Opin. Lipidol.* **1999**, *10*, 151–159.

(112) Jacobsson, A.; Stadler, U.; Glotzer, M. A.; Kozak, L. P. Mitochondrial uncoupling protein from mouse brown fat. Molecular cloning, genetic mapping, and mRNA expression. *J. Biol. Chem.* **1985**, *260*, 16250–16254.

(113) Cabrero, A.; Llaverias, G.; Roglans, N.; Alegret, M.; Sanchez, R.; Adzet, T.; Laguna, J. C.; Vazquez, M. Uncoupling protein-3 mRNA levels are increased in white adipose tissue and skeletal muscle of bezafibrate-treated rats. *Biochem. Biophys. Res. Commun.* **1999**, *260*, 547–556.

(114) Brun, S.; Carmona, M. C.; Mampel, T.; Vinas, O.; Giralt, M.; Iglesias, R.; Villarroya, F. Activators of peroxisome proliferator-activated receptor-α induce the expression of the uncoupling protein-3 gene in skeletal muscle: a potential mechanism for the lipid intake-dependent activation of uncoupling protein-3 gene expression at birth. *Diabetes* **1999**, *48*, 1217–1222.

(115) Krook, A.; Digby, J.; O'Rahilly, S.; Zierath, J. R.; Wallberg-Henriksson, H. Uncoupling protein 3 is reduced in skeletal muscle of NIDDM patients. *Diabetes* **1998**, *47*, 1528–1531.

(116) Acin, A.; Rodriguez, M.; Rique, H.; Canet, E.; Boutin, J. A.; Galizzi, J.-P. Cloning and characterization of the 5′ flanking region of the human uncoupling protein 3 (*UCP3*) gene. *Biochem. Biophys. Res. Commun.* **1999**, *258*, 278–283.

(117) Teruel, T.; Clapham, J. C.; Smith, S. A. PPARα activation by Wy 14643 induces transactivation of the rat *UCP-1* promoter without increasing UCP-1 mRNA levels and attenuates PPARγ-mediated increases in UCP-1 mRNA levels induced by rosiglitazone in fetal rat brown adipocytes. *Biochem. Biophys. Res. Commun.* **1999**, *264*, 311–315.

(118) Kobayashi, M.; Shigeta, Y.; Hirata, Y.; Omori, Y.; Sakamoto, N.; Nambu, S.; Baba, S. Improvement of glucose tolerance in NIDDM by clofibrate. Randomized double-blind study. *Diabetes Care* **1988**, *11*, 495–499.

(119) Jones, I. R.; Swai, A.; Taylor, R.; Miller, M.; Laker, M. F.; Alberti, K. G. Lowering of plasma glucose concentrations with bezafibrate in patients with moderately controlled NIDDM. *Diabetes Care* **1990**, *13*, 855–863.

(120) Inoue, I.; Takahashi, K.; Katayama, S.; Akabane, S.; Negishi, K.; Suzuki, M.; Ishii, J.; Kawazu, S. Improvement of glucose tolerance by bezafibrate in nonobese patients with hyperlipidemia and impaired glucose tolerance. *Diabetes Res. Clin. Pract.* **1994**, *25*, 199–205.

(121) Ruyter, B.; Andersen, O.; Dehli, A.; Ostlund Farrants, A.-K.; Gjoen, T.; Thomassen, M. S. Peroxisome proliferator activated receptors in Atlantic salmon (*Salmo salar*): effects on PPAR transcription and acyl-CoA oxidase activity in hepatocytes by peroxisome proliferators and fatty acids. *Biochim. Biophys. Acta* **1997**, *1348*, 331–338.

(122) Zhu, Y.; Alvares, K.; Huang, Q.; Rao, M. S.; Reddy, J. K. Cloning of a new member of the peroxisome proliferator-activated receptor gene family from mouse liver. *J. Biol. Chem.* **1993**, *268*, 26817–26820.

(123) Aperlo, C.; Pognonec, P.; Saladin, R.; Auwerx, J.; Boulukos, K. E. cDNA cloning and characterization of the transcriptional activities of the hamster peroxisome proliferator-activated receptor haPPARγ. *Gene* **1995**, *162*, 297–302.

(124) Houseknecht, K. L.; Bidwell, C. A.; Portocarrero, C. P.; Spurlock, M. E. Expression and cDNA cloning of porcine peroxisome proliferator-activated receptor γ (PPARγ). *Gene* **1998**, *225*, 89–96.

(125) Hotta, K.; Gustafson, T. A.; Yoshioka, S.; Ortmeyer, H. K.; Bodkin, N. L.; Hansen, B. C. Relationships of PPARγ and PPARγ2 mRNA levels to obesity, diabetes and hyperinsulinemia in rhesus monkeys. *Int. J. Obes.* **1998**, *22*, 1000–1010.

(126) Greene, M. E.; Blumberg, B.; McBride, O. W.; Yi, H. F.; Kronquist, K.; Kwan, K.; Hsieh, L.; Greene, G.; Nimer, S. D. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expression* **1995**, *4*, 281–299.

(127) Elbrecht, A.; Chen, Y.; Cullinan, C. A.; Hayes, N.; Leibowitz, M. D.; Moller, D. E.; Berger, J. Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors γ1 and γ2. *Biochem. Biophys. Res. Commun.* **1996**, *224*, 431–437.

(128) Lambe, K. G.; Tugwood, J. D. A human peroxisome-proliferator-activated receptor-γ is activated by inducers of adipogenesis, including thiazolidinedione drugs. *Eur. J. Biochem.* **1996**, *239*, 1–7.

(129) Beamer, B. A.; Negri, C.; Yen, C.-J.; Gavrilova, O.; Rumberger, J. M.; Durcan, M. J.; Yarnall, D. P.; Hawkins, A. L.; Griffin, C. A.; Burns, D. K.; Roth, J.; Reitman, M.; Shuldiner, A. R. Chromosomal localization and partial genomic structure of the human peroxisome proliferator activated receptor-γ (*hPPARy*) gene. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 756–759.

(130) Fajas, L.; Auboeuf, D.; Raspe, E.; Schoonjans, K.; Lefebvre, A.-M.; Saladin, R.; Najib, J.; Laville, M.; Fruchart, J.-C.; Deeb, S.; Vidal-Puig, A.; Flier, J.; Briggs, M. R.; Staels, B.; Vidal, H.; Auwerx, J. The organization, promoter analysis, and expression of the human *PPARy* gene. *J. Biol. Chem.* **1997**, *272*, 18779–18789.

(131) Mukherjee, R.; Jow, L.; Croston, G. E.; Paterniti, J. R., Jr. Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonists. *J. Biol. Chem.* **1997**, *272*, 8071–8076.

(132) Fajas, L.; Fruchart, J.-C.; Auwerx, J. PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter. *FEBS Lett.* **1998**, *438*, 55–60.

(133) Ricote, M.; Huang, J.; Fajas, L.; Li, A.; Welch, J.; Najib, J.; Witztum, J. L.; Auwerx, J.; Palinski, W.; Glass, C. K. Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7614–7619.

(134) Chawla, A.; Schwarz, E. J.; Dimaculangan, D. D.; Lazar, M. A. Peroxisome proliferator-activated receptor (PPAR) γ: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* **1994**, *135*, 798–800.

(135) Tontonoz, P.; Hu, E.; Spiegelman, B. M. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell* **1994**, *79*, 1147–1156.

(136) Tontonoz, P.; Hu, E.; Graves, R. A.; Budavari, A. I.; Spiegelman, B. M. mPPARγ2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* **1994**, *8*, 1224–1234.

(137) Saladin, R.; Fajas, L.; Dana, S.; Halvorsen, Y.-D.; Auwerx, J.; Briggs, M. Differential regulation of peroxisome proliferator activated receptor γ1 (PPARγ1) and PPARγ2 messenger RNA expression in the early stages of adipogenesis. *Cell Growth Differ.* **1999**, *10*, 43–48.

(138) Werman, A.; Hollenberg, A.; Solanes, G.; Bjorbaek, C.; Vidal-Puig, A. J.; Flier, J. S. Ligand-independent activation domain in the N terminus of peroxisome proliferator-activated receptor γ (PPARγ) Differential activity of PPARγ1 and −2 isoforms and influence of insulin. *J. Biol. Chem.* **1997**, *272*, 20230–20235.

(139) Fajas, L.; Fruchart, J.-C.; Auwerx, J. Transcriptional control of adipogenesis. *Curr. Opin. Cell Biol.* **1998**, *10*, 165–173.

(140) Spiegelman, B. M.; Flier, J. S. Adipogenesis and obesity: rounding out the big picture. *Cell* **1996**, *87*, 377–389.

(141) Tontonoz, P.; Hu, E.; Devine, J.; Beale, E. G.; Spiegelman, B. M. PPAR-γ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol. Cell. Biol.* **1995**, *15*, 351–357.

(142) Schoonjans, K.; Watanabe, M.; Suzuki, H.; Mahfoudi, A.; Krey, G.; Wahli, W.; Grimaldi, P.; Staels, B.; Yamamoto, T.; Auwerx, J. Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. *J. Biol. Chem.* **1995**, *270*, 19269–19276.

(143) Martin, G.; Schoonjans, K.; Lefebvre, A.-M.; Staels, B.; Auwerx, J. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARα and PPARγ activators. *J. Biol. Chem.* **1997**, *272*, 28210–28217.

(144) Sfeir, Z.; Ibrahimi, A.; Amri, E.; Grimaldi, P.; Abumrad, N. Regulation of FAT/CD36 gene expression: further evidence in support of a role of the protein in fatty acid binding/transport. *Prostaglandins Leukotrienes Essent. Fatty Acids* **1997**, *57*, 17–21.

(145) Kallen, C. B.; Lazar, M. A. Antidiabetic thiazolidinediones inhibit leptin (*ob*) gene expression in 3T3-L1 adipocytes. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5793–5796.

(146) De Vos, P.; Lefebvre, A.-M.; Miller, S. G.; Guerre-Millo, M.; Wong, K.; Saladin, R.; Hamann, L. G.; Staels, B.; Briggs, M. R.; Auwerx, J. Thiazolidinediones repress *ob* gene expression in rodents via activation of peroxisome proliferator-activated receptor γ. *J. Clin. Invest.* **1996**, *98*, 1004–1009.

(147) Hofmann, C.; Lorenz, K.; Braithwaite, S. S.; Colca, J. R.; Palazuk, B. J.; Hotamisligil, G. S.; Spiegelman, B. M. Altered gene expression for tumor necrosis factor-α and its receptors during drug and dietary modulation of insulin resistance. *Endocrinology* **1994**, *134*, 264–270.

(148) Aubert, J.; Champigny, O.; Saint-Marc, P.; Negrel, R.; Collins, S.; Ricquier, D.; Ailhaud, G. Up-regulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells. *Biochem. Biophys. Res. Commun.* **1997**, *238*, 606–611.

(149) Cambon, B.; Reyne, Y.; Nougues, J. In vitro induction of UCP1 mRNA in preadipocytes from rabbit considered as a model of large mammals brown adipose tissue development: importance of PPARγ agonists for cells isolated in the postnatal period. *Mol. Cell. Endocrinol.* **1998**, *146*, 49–58.

(150) Camirand, A.; Marie, V.; Rabelo, R.; Silva, J. E. Thiazolidinediones stimulate uncoupling protein-2 expression in cell lines representing white and brown adipose tissues and skeletal muscle. *Endocrinology* **1998**, *139*, 428–431.

(151) Sears, I. B.; MacGinnitie, M. A.; Kovacs, L. G.; Graves, R. A. Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor γ. *Mol. Cell. Biol.* **1996**, *16*, 3410–3419.

(152) Tai, T.-A. C.; Jennermann, C.; Brown, K. K.; Oliver, B. B.; MacGinnitie, M. A.; Wilkison, W. O.; Brown, H. R.; Lehmann, J. M.; Kliewer, S. A.; Morris, D. C.; Graves, R. A. Activation of the nuclear receptor peroxisome proliferator-activated receptor γ promotes brown adipocyte differentiation. *J. Biol. Chem.* **1996**, *271*, 29909–29914.

(153) Kelly, L. J.; Vicario, P. P.; Thompson, G. M.; Candelore, M. R.; Doebber, T. W.; Ventre, J.; Wu, M. S.; Meurer, R.; Forrest, M. J.; Conner, M. W.; Cascieri, M. A.; Moller, D. E. Peroxisome proliferator-activated receptors γ and α mediate in vivo regulation of uncoupling protein (*UCP-1, UCP-2, UCP-3*) gene expression. *Endocrinology* **1998**, *139*, 4920–4927.

(154) Ribon, V.; Johnson, J. H.; Camp, H. S.; Saltiel, A. R. Thiazolidinediones and insulin resistance: peroxisome proliferator-activated receptor γ activation stimulates expression of the *CAP* gene. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14751–14756.

(155) Hulin, B.; McCarthy, P. A.; Gibbs, E. M. The glitazone family of antidiabetic agents. *Curr. Pharm. Des.* **1996**, *2*, 85–102.

(156) Kletzien, R. F.; Clarke, S. D.; Ulrich, R. G. Enhancement of adipocyte differentiation by an insulin-sensitizing agent. *Mol. Pharmacol.* **1992**, *41*, 393–398.

(157) Ibrahimi, A.; Teboul, L.; Gaillard, D.; Amri, E. Z.; Ailhaud, G.; Young, P.; Cawthorne, M. A.; Grimaldi, P. A. Evidence for a common mechanism of action for fatty acids and thiazolidinedione antidiabetic agents on gene expression in preadipose cells. *Mol. Pharmacol.* **1994**, *46*, 1070–1076.

(158) Harris, P. K. W.; Kletzien, R. F. Localization of a pioglitazone response element in the adipocyte fatty acid-binding protein gene. *Mol. Pharmacol.* **1994**, *45*, 439–445.

(159) Forman, B. M.; Tontonoz, P.; Chen, J.; Brun, R. P.; Spiegelman, B. M.; Evans, R. M. 15-Deoxy-Δ^{12,14}-prostaglandin J$_2$ is a ligand for the adipocyte determination factor PPARγ. *Cell* **1995**, *83*, 803–812.

(160) Willson, T. M.; Cobb, J. E.; Cowan, D. J.; Wiethe, R. W.; Correa, I. D.; Prakash, S. R.; Beck, K. D.; Moore, L. B.; Kliewer, S. A.; Lehmann, J. M. The structure−activity relationship between peroxisome proliferator-activated receptor γ agonism and the anti-hyperglycemic activity of thiazolidinediones. *J. Med. Chem.* **1996**, *39*, 665–668.

(161) Murakami, K.; Tobe, K.; Ide, T.; Mochizuki, T.; Ohashi, M.; Akanuma, Y.; Yazaki, Y.; Kadowaki, T. A novel insulin sensitizer acts as a coligand for peroxisome proliferator-activated receptor-α (PPAR-α) and PPAR-γ: effect of PPAR-α activation on abnormal lipid metabolism in liver of Zucker fatty rats. *Diabetes* **1998**, *47*, 1841–1847.

(162) Nomura, M.; Kinoshita, S.; Satoh, H.; Maeda, T.; Murakami, K.; Tsunoda, M.; Miyachi, H.; Awano, K. (3-Substituted benzyl)-thiazolidine-2,4-diones as structurally new antihyperglycemic agents. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 533–538.

(163) Ide, T.; Murakami, K.; Tobe, K.; Mochizuki, T.; Ohashi, M.; Akanuma, Y.; Kadowaki, T.; Yazaki, Y. Effects of PPARα activation on liver lipid metabolism in Zucker fatty rats. *Diabetes Front* **1998**, *9*, 345–346.

(164) Shibata, T.; Matsui, K.; Nagao, K.; Shinkai, H.; Yonemori, F.; Wakitani, K. Pharmacological profiles of a novel oral antidiabetic agent, JTT-501, an isoxazolidinedione derivative. *Eur. J. Pharmacol.* **1999**, *364*, 211–219.

(165) Shinkai, H.; Onogi, S.; Tanaka, M.; Shibata, T.; Iwao, M.; Wakitani, K.; Uchida, I. Isoxazolidine-3,5-dione and noncyclic 1,3-dicarbonyl compounds as hypoglycemic agents. *J. Med. Chem.* **1998**, *41*, 1927–1933.

(166) Upton, R.; Widdowson, P. S.; Ishii, S.; Tanaka, H.; Williams, G. Improved metabolic status and insulin sensitivity in obese fatty (fa/fa) Zucker rats and Zucker Diabetic Fatty (ZDF) rats treated with the thiazolidinedione, MCC-555. *Br. J. Pharmacol.* **1998**, *125*, 1708–1714.

(167) Pickavance, L.; Widdowson, P. S.; King, P.; Ishii, S.; Tanaka, H.; Williams, G. The development of overt diabetes in young Zucker Diabetic Fatty (ZDF) rats and the effects of chronic MCC-555 treatment. *Br. J. Pharmacol.* **1998**, *125*, 767–770.

(168) Reginato, M. J.; Bailey, S. T.; Krakow, S. L.; Minami, C.; Ishii, S.; Tanaka, H.; Lazar, M. A. A potent antidiabetic thiazolidinedione with unique peroxisome proliferator-activated receptor γ-activating properties. *J. Biol. Chem.* **1998**, *273*, 32679–32684.

(169) Reddy, K. A.; Lohray, B. B.; Bhushan, V.; Reddy, A. S.; Rao Mamidi, N. V. S.; Reddy, P. P.; Saibaba, V.; Reddy, N. J.; Suryaprakash, A.; Misra, P.; Vikramadithyan, R. K.; Rajagopalan, R. Novel antidiabetic and hypolipidemic agents. 5. Hydroxyl versus benzyloxy containing chroman derivatives. *J. Med. Chem.* **1999**, *42*, 3265–3278.

(170) Lohray, B. B.; Bhushan, V.; Reddy, A. S.; Rao, P. B.; Reddy, N. J.; Harikishore, P.; Haritha, N.; Vikramadityan, R. K.; Chakrabarti, R.; Rajagopalan, R.; Katneni, K. Novel euglycemic and hypolipidemic agents. 4. Pyridyl- and quinolinyl-containing thiazolidinediones. *J. Med. Chem.* **1999**, *42*, 2569–2581.

(171) Sato, M.; Grese, T. A.; Dodge, J. A.; Bryant, H. U.; Turner, C. H. Emerging therapies for the prevention or treatment of postmenopausal osteoporosis. *J. Med. Chem.* **1999**, *42*, 1–24.

(172) Sohda, T.; Mizuno, K.; Kawamatsu, Y. Studies on antidiabetic agents. VI. Asymmetric transformation of (±)-5-[4-(1-methylcyclohexylmethoxy)benzyl]-2,4-thiazolidinedione (ciglitazone) with optically active 1-phenylethylamines. *Chem. Pharm. Bull.* **1984**, *32*, 4460–4465.

(173) Parks, D. J.; Tomkinson, N. C. O.; Villeneuve, M. S.; Blanchard, S. G.; Willson, T. M. Differential activity of rosiglitazone enantiomers at PPARγ. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3657–3658.

(174) Buckle, D. R.; Cantello, B. C. C.; Cawthorne, M. A.; Coyle, P. J.; Dean, D. K.; Faller, A.; Haigh, D.; Hindley, R. M.; Jefcott, L. J.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Smith, D. G.; Smith, S. A. Non-thiazolidinedione antihyperglycemic agents. 1: α-Heteroatom substituted β-phenylpropanoic acids. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2121–2126.

(175) Buckle, D. R.; Cantello, B. C. C.; Cawthorne, M. A.; Coyle, P. J.; Dean, D. K.; Faller, A.; Haigh, D.; Hindley, R. M.; Jefcott, L. J.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Smith, D. G.; Smith, S. A. Non-thiazolidinedione antihyperglycemic agents. 2: α-Carbon substituted β-phenylpropanoic acids. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2127–2130.

(176) Haigh, D.; Allen, G.; Birrell, H. C.; Buckle, D. R.; Cantello, B. C. C.; Eggleston, D. S.; Haltiwanger, R. C.; Holder, J. C.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Sime, J. T.; Smith, S. A.; Sweeney, J. D. Non-thiazolidinedione antihyperglycaemic agents. Part 3: The effects of stereochemistry on the potency of α-methoxy-β-phenylpropanoic acids. *Bioorg. Med. Chem.* **1999**, *7*, 821–830.

(177) Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K.; Cobb, J. E.; Collins, J. L.; Harrington, W. W., Jr.; Hashim, M. A.; Hull-Ryde, E. A.; Kaldor, I.; Kliewer, S. A.; Lake, D. H.; Leesnitzer, L. M.; Lehmann, J. M.; Lenhard, J. M.; Orband-Miller, L. A.; Miller, J. F.; Mook, R. A.; Noble, S. A.; Oliver, W.; Parks, D. J.; Plunket, K. D.; Szewczyk, J. R.; Willson, T. M. *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 1. Discovery of a novel series of potent antihyperglycemic and antihyperlipidemic agents. *J. Med. Chem.* **1998**, *41*, 5020–5036.

(178) Collins, J. L.; Blanchard, S. G.; Boswell, G. E.; Charifson, P. S.; Cobb, J. E.; Henke, B. R.; Hull-Ryde, E. A.; Kazmierski, W. M.; Lake, D. H.; Leesnitzer, L. M.; Lehmann, J.; Lenhard, J. M.; Orband-Miller, L. A.; Gray-Nunez, Y.; Parks, D. J.; Plunkett, K. D.; Tong, W.-Q. *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 2. Structure–activity relationship and optimization of the phenyl alkyl ether moiety. *J. Med. Chem.* **1998**, *41*, 5037–5054.

(179) Cobb, J. E.; Blanchard, S. G.; Boswell, E. G.; Brown, K. K.; Charifson, P. S.; Cooper, J. P.; Collins, J. L.; Dezube, M.; Henke, B. R.; Hull-Ryde, E. A.; Lake, D. H.; Lenhard, J. M.; Oliver, W., Jr.; Oplinger, J.; Pentti, M.; Parks, D. J.; Plunket, K. D.; Tong, W.-Q. *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 3. Structure–activity relationship and optimization of the *N*-aryl substituent. *J. Med. Chem.* **1998**, *41*, 5055–5069.

(180) Brown, K. K.; Henke, B. R.; Blanchard, S. G.; Cobb, J. E.; Kaldor, I.; Kliewer, S. A.; Lehmann, J. M.; Lenhard, J. M.; Harrington, W.; Novak, P. J.; Binz, J.; Hashim, M. A.; Oliver, W. O.; Brown, H. R.; Parks, D. J.; Plunket, K. D.; Tong, T.; Menius, A.; Adkison, K. K.; Noble, S. A.; Willson, T. M. A novel *N*-aryl tyrosine activator of peroxisome proliferator-activated receptor-γ reverses the diabetic phenotype of the Zucker diabetic fatty rat. *Diabetes* **1999**, *48*, 1415–1142.

(181) Henke, B. R.; Adkison, K. K.; Blanchard, S. G.; Leesnitzer, L. M.; Robert A. Mook, J.; Kelli D. Plunket; Ray, J. A.; Roberson, C.; Unwalla, R.; Willson, T. M. Synthesis and biological activity of a novel series of indole-derived PPARγ agonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3329–3334.

(182) Lehmann, J. M.; Lenhard, J. M.; Oliver, B. B.; Ringold, G. M.; Kliewer, S. A. Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other nonsteroidal antiinflammatory drugs. *J. Biol. Chem.* **1997**, *272*, 3406–3410.

(183) Verrando, P.; Negrel, R.; Grimaldi, P.; Murphy, M.; Ailhaud, G. Differentiation of ob 17 preadipocytes to adipocytes. Triggering effects of clofenapate and indomethacin. *Biochim. Biophys. Acta* **1981**, *663*, 255–265.

(184) Knight, D. M.; Chapman, A. B.; Navre, M.; Drinkwater, L.; Bruno, J. J.; Ringold, G. M. Requirements of triggering of adipocyte differentiation by glucocorticoids and indomethacin. *Mol. Endocrinol.* **1987**, *1*, 36–43.

(185) Huang, J. T.; Welch, J. S.; Ricote, M.; Binder, C. J.; Willson, T. M.; Kelly, C.; Witztum, J. L.; Funk, C. D.; Conrad, D.; Glass, C. K. Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase. *Nature* **1999**, *400*, 378–382.

(186) Nagy, L.; Tontonoz, P.; Alvarez, J. G. A.; Chen, H.; Evans, R. M. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. *Cell* **1998**, *93*, 229–240.

(187) Kliewer, S. A.; Lenhard, J. M.; Willson, T. M.; Patel, I.; Morris, D. C.; Lehmann, J. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell* **1995**, *83*, 813–819.

(188) Satoh, T.; Furuta, K.; Suzuki, M.; Watanabe, Y. Prostaglandin J2 and its metabolites promote neurite outgrowth induced by nerve growth factor in PC12 cells. *Biochem. Biophys. Res. Commun.* **1999**, *258*, 50–53.

(189) Negishi, M.; Koizumi, T.; Ichikawa, A. Biological actions of Δ^{12}-prostaglandin J2. *J. Lipid Mediators Cell Signalling* **1995**, *12*, 443–448.

(190) Fukushima, M. Biological activities and mechanisms of action of PGJ2 and related compounds: an update. *Prostaglandins Leukotrienes Essent. Fatty Acids* **1992**, *47*, 1–12.

(191) Narumiya, S.; Fukushima, M. Cyclopentenone prostaglandins: antiproliferative and antiviral actions and their molecular mechanism. *Dev. Oncol.* **1991**, *67*, 439–448.

(192) Oberfield, J. L.; Collins, J. L.; Holmes, C. P.; Goreham, D. M.; Cooper, J. P.; Cobb, J. E.; Lenhard, J. M.; Hull-Ryde, E. A.; Mohr, C. P.; Blanchard, S. G.; Parks, D. J.; Moore, L. B.; Lehmann, J. M.; Plunket, K.; Miller, A. B.; Milburn, M. V.; Kliewer, S. A.; Willson, T. M. A peroxisome proliferator-activated receptor γ ligand inhibits adipocyte differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6102–6106.

(193) Weatherman, R. V.; Fletterick, R. J.; Scanlan, T. S. Nuclear-receptor ligands and ligand-binding domains. *Annu. Rev. Biochem.* **1999**, *68*, 559–581.

(194) Spiegelman, B. M. PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes* **1998**, *47*, 507–514.

(195) Grossman, S. L.; Lessem, J. Mechanisms and clinical effects of thiazolidinediones. *Expert Opin. Invest. Drugs* **1997**, *6*, 1025–1040.

(196) Chaiken, R. L.; Eckert-Norton, M.; Pasmantier, R.; Boden, G.; Ryan, I.; Gelfand, R. A.; Lebovitz, H. E. Metabolic effects of darglitazone, an insulin sensitizer, in NIDDM subjects. *Diabetologia* **1995**, *38*, 1307–1312.

(197) Colca, J. R.; Morton, D. R. In *New Antidiabetic Drugs*; Bailey, C. J., Flatt, P. R., Eds.; Smith-Gordon: New York, 1990; pp 255–261.

(198) Ikeda, H.; Taketomi, S.; Sugiyama, Y.; Shimura, Y.; Sohda, T.; Meguro, K.; Fujita, T. Effects of pioglitazone on glucose and lipid metabolism in normal and insulin resistant animals. *Arzneim.-Forsch.* **1990**, *40*, 156–162.

(199) Horikoshi, H.; Yoshioka, T.; Kawasaki, T.; Nakamura, K.-I.; Matsunuma, N.; Yamaguchi, K.; Sasahara, K. Troglitazone (CS-045), a new antidiabetic drug. *Annu. Rep. Sankyo Res. Lab.* **1994**, *46*, 1–57.

(200) Prigeon, R. L.; Kahn, S. E.; Porte, D., Jr. Effect of troglitazone on β cell function, insulin sensitivity, and glycemic control in subjects with type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 819–823.

(201) Fonseca, V. A.; Valiquett, T. R.; Huang, S. M.; Ghazzi, M. N.; Whitcomb, R. W. Troglitazone monotherapy improves glycemic control in patients with type 2 diabetes mellitus: a randomized, controlled study. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 3169–3176.

(202) Spencer, C. M.; Markham, A. Troglitazone. *Drugs* **1997**, *54*, 89–101.

(203) Kumar, S.; Boulton, A. J. M.; Beck-Nielsen, H.; Berthezene, F.; Muggeo, M.; Persson, B.; Spinas, G. A.; Donoghue, S.; Lettis, S.; Stewart-Long, P. Troglitazone, an insulin action enhancer, improves metabolic control in NIDDM patients. *Diabetologia* **1996**, *39*, 701–709.

(204) Ghazzi, M. N.; Perez, J. E.; Antonucci, T. K.; Driscoll, J. H.; Huang, S. M.; Faja, B. W.; Whitcomb, R. W. Cardiac and glycemic benefits of troglitazone treatment in NIDDM. *Diabetes* **1997,** *46,* 433–439.

(205) Wang, Q.; Dryden, S.; Frankish, H. M.; Bing, C.; Pickavance, L.; Hopkins, D.; Buckingham, R.; Williams, G. Increased feeding in fatty Zucker rats by the thiazolidinedione BRL 49653 (rosiglitazone) and the possible involvement of leptin and hypothalamic neuropeptide Y. *Br. J. Pharmacol.* **1997,** *122,* 1405–1410.

(206) Hallakou, S.; Doare, L.; Foufelle, F.; Kergoat, M.; Guerre-Millo, M.; Berthault, M.-F.; Dugail, I.; Morin, J.; Auwerx, J.; Ferre, P. Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker fa/fa rat. *Diabetes* **1997,** *46,* 1393–1399.

(207) Gimble, J. M.; Robinson, C. E.; Wu, X.; Kelly, K. A.; Rodriguez, B. R.; Kliewer, S. A.; Lehmann, J. M.; Morris, D. C. Peroxisome proliferator-activated receptor-γ activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. *Mol. Pharmacol.* **1996,** *50,* 1087–1094.

(208) Watkins, P. B.; Whitcomb, R. W. Hepatic dysfunction associated with troglitazone. *N. Engl. J. Med.* **1998,** *338,* 916–917.

(209) Jones, S. A.; Moore, L. B.; Shenk, J. L.; Wisely, G. B.; Hamilton, G. A.; McKee, D. D.; Tomkinson, N. C. O.; LeCluyse, E. L.; Lambert, M. H.; Willson, T. M.; Kliewer, S. A.; Moore, J. T. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol. Endocrinol.* **2000,** *14,* 27–39.

(210) Kliewer, S. A.; Moore, J. T.; Wade, L.; Staudinger, J. L.; Watson, M. A.; Jones, S. A.; McKee, D. D.; Oliver, B. B.; Willson, T. M.; Zetterstrom, R. H.; Perlmann, T.; Lehmann, J. M. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **1998,** *92,* 73–82.

(211) Lehmann, J. M.; McKee, D. D.; Watson, M. A.; Willson, T. M.; Moore, J. T.; Kliewer, S. A. The human orphan nuclear receptor PXR is activated by compounds that regulate *CYP3A4* gene expression and cause drug interactions. *J. Clin. Invest.* **1998,** *102,* 1016–1023.

(212) Bertilsson, G.; Heidrich, J.; Svensson, K.; Asman, M.; Jendeberg, L.; Sydow-Backman, M.; Ohlsson, R.; Postlind, H.; Blomquist, P.; Berkenstam, A. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl. Acad. Sci. U.S.A.* **1998,** *95,* 12208–12213.

(213) Blumberg, B.; Sabbagh, W., Jr.; Juguilon, H.; Bolado, J., Jr.; Van Meter, C. M.; Ong, E. S.; Evans, R. M. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev.* **1998,** *12,* 3195–3205.

(214) Michalets, E. L. Update: clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy* **1998,** *18,* 84–112.

(215) Ramachandran, V.; Kostrubsky, V. E.; Komoroski, B. J.; Zhang, S.; Dorko, K.; Esplen, J. E.; Strom, S. C.; Venkataramanan, R. Troglitazone increases cytochrome P-450 3A protein and activity in primary cultures of human hepatocytes. *Drug Metab. Dispos.* **1999,** *27,* 1194–1199.

(216) Yamazaki, H.; Shibata, A.; Suzuki, M.; Nakajima, M.; Shimada, N.; Guengerich, F. P.; Yokoi, T. Oxidation of troglitazone to a quinone-type metabolite catalyzed by cytochrome P-450 2C8 and P-450 3A4 in human liver microsomes. *Drug Metab. Dispos.* **1999,** *27,* 1260–1266.

(217) Pumford, N. R.; Halmes, N. C. Protein targets of xenobiotic reactive intermediates. *Annu. Rev. Pharmacol. Toxicol.* **1997,** *37,* 91–117.

(218) Mukherjee, R.; Davies, P. J. A.; Crombie, D. L.; Bischoff, E. D.; Cesario, R. M.; Jow, L.; Hamann, L. G.; Boehm, M. F.; Mondon, C. E.; Nadzan, A. M.; Paterniti, J. R., Jr.; Heyman, R. A. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* **1997,** *386,* 407–410.

(219) Mukherjee, R.; Strasser, J.; Jow, L.; Hoener, P.; Paterniti, J. R., Jr; Heyman, R. A. RXR agonists activate PPARα-inducible genes, lower triglycerides, and raise HDL levels in vivo. *Arterioscler. Thromb. Vasc. Biol.* **1998,** *18,* 272–276.

(220) Miller, V. A.; Benedetti, F. M.; Rigas, J. R.; Verret, A. L.; Pfister, D. G.; Straus, D.; Kris, M. G.; Crisp, M.; Heyman, R.; Loewen, G. R.; Truglia, J. A.; Warrell, R. P., Jr. Initial clinical trial of a selective retinoid X receptor ligand, LGD1069. *J. Clin. Oncol.* **1997,** *15,* 790–795.

(221) Sherman, S. I.; Gopal, J.; Haugen, B. R.; Chiu, A. C.; Whaley, K.; Nowlakha, P.; Duvic, M. Central hypothyroidism associated with retinoid X receptor-selective ligands. *N. Engl. J. Med.* **1999,** *340,* 1075–1079.

(222) Rizvi, N. A.; Marshall, J. L.; Dahut, W.; Ness, E.; Truglia, J. A.; Loewen, G.; Gill, G. M.; Ulm, E. H.; Geiser, R.; Jaunakais, D.; Hawkins, M. J. A phase I study of LGD1069 in adults with advanced cancer. *Clin. Cancer Res.* **1999,** *5,* 1658–1664.

(223) Burant, C. F.; Sreenan, S.; Hirano, K.-i.; Tai, T.-A. C.; Lohmiller, J.; Lukens, J.; Davidson, N. O.; Ross, S.; Graves, R. A. Troglitazone action is independent of adipose tissue. *J. Clin. Invest.* **1997,** *100,* 2900–2908.

(224) Kruszynska, Y. T.; Mukherjee, R.; Jow, L.; Dana, S.; Paterniti, J. R., Jr.; Olefsky, J. M. Skeletal muscle peroxisome proliferator-activated receptor-γ expression in obesity and non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* **1998,** *101,* 543–548.

(225) Vidal-Puig, A. J.; Considine, R. V.; Jimenez-Linan, M.; Werman, A.; Pories, W. J.; Caro, J. F.; Flier, J. S. Peroxisome proliferator-activated receptor gene expression in human tissues: effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J. Clin. Invest.* **1997,** *99,* 2416–2422.

(226) Park, K. S.; Ciaraldi, T. P.; Abrams-Carter, L.; Mudaliar, S.; Nikoulina, S. E.; Henry, R. R. PPAR-γ gene expression is elevated in skeletal muscle of obese and type II diabetic subjects. *Diabetes* **1997,** *46,* 1230–1234.

(227) Wu, Z.; Xie, Y.; Morrison, R. F.; Bucher, N. L. R.; Farmer, S. R. PPARγ induces the insulin-dependent glucose transporter GLUT4 in the absence of C/EBPα during the conversion of 3T3 fibroblasts into adipocytes. *J. Clin. Invest.* **1998,** *101,* 22–32.

(228) Young, P. W.; Cawthorne, M. A.; Coyle, P. J.; Holder, J. C.; Holman, G. D.; Kozka, I. J.; Kirkham, D. M.; Lister, C. A.; Smith, S. A. Repeat treatment of obese mice with BRL 49653, a new and potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. *Diabetes* **1995,** *44,* 1087–1092.

(229) Fleury, C.; Neverova, M.; Collins, S.; Raimbault, S.; Champigny, O.; Levi-Meyrueis, C.; Bouillaud, F.; Seldin, M. F.; Surwit, R. S.; Ricquier, D.; Warden, C. H. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* **1997,** *15,* 269–272.

(230) Randle, P. J. Regulatory interactions between lipids and carbohydrates: the Glucose Fatty Acid Cycle after 35 years. *Diabetes/Metab. Rev.* **1998,** *14,* 263–283.

(231) Martin, G.; Schoonjans, K.; Staels, B.; Auwerx, J. PPARγ activators improve glucose homeostasis by stimulating fatty acid uptake in the adipocytes. *Atherosclerosis* **1998,** *137,* S75–S80.

(232) Sreenan, S.; Keck, S.; Fuller, T.; Cockburn, B.; Burant, C. F. Effects of troglitazone on substrate storage and utilization in insulin-resistant rats. *Am. J. Physiol.* **1999,** *276,* E1119–E1129.

(233) Tontonoz, P.; Nagy, L.; Alvarez, J. G. A.; Thomazy, V. A.; Evans, R. M. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* **1998,** *93,* 241–252.

(234) Aitman, T. J.; Glazier, A. M.; Wallace, C. A.; Cooper, L. D.; Norsworthy, P. J.; Wahid, F. N.; Al-Majali, K. M.; Trembling, P. M.; Mann, C. J.; Shoulders, C. C.; Graf, D.; Lezin, E. S.; Kurtz, T. W.; Kren, V.; Pravenec, M.; Ibrahimi, A.; Abumrad, N. A.; Stanton, L. W.; Scott, J. Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat. Genet.* **1999,** *21,* 76–83.

(235) Hallakou, S.; Foufelle, F.; Doare, L.; Kergoat, M.; Ferre, P. Pioglitazone-induced increase of insulin sensitivity in the muscles of the obese Zucker fa/fa rat cannot be explained by local adipocyte differentiation. *Diabetologia* **1998,** *41,* 963–968.

(236) Okuno, A.; Tamemoto, H.; Tobe, K.; Ueki, K.; Mori, Y.; Iwamoto, K.; Umesono, K.; Akanuma, Y.; Fujiwara, T.; Horikoshi, H.; Yazaki, Y.; Kadowaki, T. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J. Clin. Invest.* **1998,** *101,* 1354–1361.

(237) Hotamisligil, G. S.; Peraldi, P.; Spiegelman, B. M. The molecular link between obesity and diabetes. *Curr. Opin. Endocrinol. Diabetes* **1996,** *3,* 16–23.

(238) Peraldi, P.; Xu, M.; Spiegelman, B. M. Thiazolidinediones block tumor necrosis factor-α-induced inhibition of insulin signaling. *J. Clin. Invest.* **1997,** *100,* 1863–1869.

(239) Miles, P. D. G.; Romeo, O. M.; Higo, K.; Cohen, A.; Rafaat, K.; Olefsky, J. M. TNF-α-induced insulin resistance in vivo and its prevention by troglitazone. *Diabetes* **1997,** *46,* 1678–1683.

(240) Rebrin, K.; Steil, G. M.; Mittelman, S. D.; Bergman, R. N. Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. *J. Clin. Invest.* **1996,** *98,* 741–749.

(241) Rebrin, K.; Steil, G. M.; Getty, L.; Bergman, R. N. Free fatty acid as a link to the regulation of hepatic glucose output by peripheral insulin. *Diabetes* **1995,** *44,* 1038–1045.

(242) Digby, J. E.; Montague, C. T.; Sewter, C. P.; Sanders, L.; Wilkison, W. O.; O'Rahilly, S.; Prins, J. B. Thiazolidinedione exposure increases the expression of uncoupling protein 1 in cultured human preadipocytes. *Diabetes* **1998,** *47,* 138–141.

(243) Ristow, M.; Muller-Wieland, D.; Pfeiffer, A.; Krone, W.; Kahn, C. R. Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *N. Engl. J. Med.* **1998,** *339,* 953–959.

(244) Meirhaeghe, A.; Fajas, L.; Helbecque, N.; Cottel, D.; Lebel, P.; Dallongeville, J.; Deeb, S.; Auwerx, J.; Amouyell, P. A genetic polymorphism of the peroxisome proliferator-activated receptor γ gene influences plasma leptin levels in obese humans. *Hum. Mol. Genet.* **1998,** *7,* 435–440.

(245) Yen, C.-J.; Beamer, B. A.; Negri, C.; Silver, K.; Brown, K. A.; Yarnall, D. P.; Burns, D. K.; Roth, J.; Shuldiner, A. R. Molecular scanning of the human peroxisome proliferator activated receptor γ (hPPARγ) gene in diabetic caucasians: identification of a Pro¹²Ala PPARγ2 missense mutation. *Biochem. Biophys. Res. Commun.* **1997**, *241*, 270–274.

(246) Deeb, S. S.; Fajas, L.; Nemoto, M.; Pihlajamaki, J.; Mykkanen, L.; Kuusisto, J.; Laakso, M.; Fujimoto, W.; Auwerx, J. A Pro¹²-Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat. Genet.* **1998**, *20*, 284–287.

(247) Valve, R.; Sivenius, K.; Miettinen, R.; Pihlajamaki, J.; Rissanen, A.; Deeb, S. S.; Auwerx, J.; Uusitupa, M.; Laakso, M. Two polymorphisms in the peroxisome proliferator-activated receptor-γ gene are associated with severe overweight among obese women. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 3708–3712.

(248) Mori, Y.; Kim-Motoyama, H.; Katakura, T.; Yasuda, K.; Kadowaki, H.; Beamer, B. A.; Shuldiner, A. R.; Akanuma, Y.; Yazaki, Y.; Kadowaki, T. Effect of the Pro¹²Ala variant of the human peroxisome proliferator-activated receptor γ2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men. *Biochem. Biophys. Res. Commun.* **1998**, *251*, 195–198.

(249) Beamer, B. A.; Yen, C.-J.; Andersen, R. E.; Muller, D.; Elahi, D.; Cheskin, L. J.; Andres, R.; Roth, J.; Shuldiner, A. R. Association of the Pro¹²Ala variant in the peroxisome proliferator-activated receptor-γ2 gene with obesity in two Caucasian populations. *Diabetes* **1998**, *47*, 1806–1808.

(250) Ek, J.; Urhammer, S. A.; Sorensen, T. I. A.; Andersen, T.; Auwerx, J.; Pedersen, O. Homozygosity of the Pro¹²Ala variant of the peroxisome proliferation-activated receptor-γ2 (PPAR-γ2). Divergent modulating effects on body mass index in obese and lean Caucasian men. *Diabetologia* **1999**, *42*, 892–895.

(251) Koch, M.; Rett, K.; Maerker, E.; Volk, A.; Haist, K.; Deninger, M.; Renn, W.; Haring, H. U. The PPARγ2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* **1999**, *42*, 758–762.

(252) Ringel, J.; Engeli, S.; Distler, A.; Sharma, A. M. Pro¹²Ala missense mutation of the peroxisome proliferator activated receptor γ and diabetes mellitus. *Biochem. Biophys. Res. Commun.* **1999**, *254*, 450–453.

(253) Hamann, A.; Munzberg, H.; Buttron, P.; Busing, B.; Hinney, A.; Mayer, H.; Siegfried, W.; Hebebrand, J.; Greten, H. Missense variants in the human peroxisome proliferator-activated receptor-γ2 gene in lean and obese subjects. *Eur. J. Endocrinol.* **1999**, *141*, 90–92.

(254) Barak, Y.; Nelson, M. C.; Ong, E. S.; Jones, Y. Z.; Ruiz-Lozano, P.; Chien, K. R.; Koder, A.; Evans, R. M. PPARγ is required for placental, cardiac, and adipose tissue development. *Mol. Cell* **1999**, *4*, 585–595.

(255) Kubota, N.; Terauchi, Y.; Miki, H.; Tamemoto, H.; Yamauchi, T.; Komeda, K.; Satoh, S.; Nakano, R.; Ishii, C.; Sugiyama, T.; Eto, K.; Tsubamoto, Y.; Okuno, A.; Murakami, K.; Sekihara, H.; Hasegawa, G.; Naito, M.; Toyoshima, Y.; Tanaka, S.; Shiota, K.; Kitamura, T.; Fujita, T.; Ezaki, O.; Aizawa, S.; Nagai, R.; Tobe, K.; Kimura, S.; Kadowaki, T. PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol. Cell* **1999**, *4*, 597–609.

(256) Rosen, E. D.; Sarraf, P.; Troy, A. E.; Bradwin, G.; Moore, K.; Milstone, D. S.; Spiegelman, B. M.; Mortensen, R. M. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. *Mol. Cell* **1999**, *4*, 611–617.

(257) Chen, D.; Garg, A. Monogenic disorders of obesity and body fat distribution. *J. Lipid Res.* **1999**, *40*, 1735–1746.

(258) Girard, J. Is leptin the link between obesity and insulin resistance? *Diabetes Metab.* **1997**, *23*, 16–24.

(259) Lowell, B. B. PPARγ: an essential regulator of adipogenesis and modulator of fat cell function. *Cell* **1999**, *99*, 239–242.

(260) Rezulin prescribing information at http://www.parke-davis.com/REZULIN.pdf.

(261) Avandia prescribing information at http://www.avandia.com/index.asp.

(262) Actos prescribing information at http://www.actos.com/pi.htm.

(263) McKeigue, P.; Berne, C.; Lithell, H. Insulin and hypertension. *Handb. Hypertens.* **1997**, *17*, 911–934.

(264) Dubey, R. K.; Zhang, H. Y.; Reddy, S. R.; Boegehold, M. A.; Kotchen, T. A. Pioglitazone attenuates hypertension and inhibits growth of renal arteriolar smooth muscle in rats. *Am. J. Physiol.* **1993**, *265*, R726–R732.

(265) Walker, A. B.; Chattington, P. D.; Buckingham, R. E.; Williams, G. The thiazolidinedione rosiglitazone (BRL-49653) lowers blood pressure and protects against impairment of endothelial function in Zucker fatty rats. *Diabetes* **1999**, *48*, 1448–1453.

(266) Yoshioka, S.; Nishino, H.; Shiraki, T.; Ikeda, K.; Koibe, H.; Okuno, A.; Wada, M.; Fujiwara, T.; Horikoshi, H. Antihypertensive effects of CS-045 treatment in obese Zucker rats. *Metab. Clin. Exp.* **1993**, *42*, 75–80.

(267) Pershadsingh, H. A.; Szollosi, J.; Benson, S.; Hyun, W. C.; Feuerstein, B. G.; Kurtz, T. W. Effects of ciglitazone on blood pressure and intracellular calcium metabolism. *Hypertension* **1993**, *21*, 1020–1023.

(268) Saku, K.; Zhang, B.; Ohta, T.; Arakawa, K. Troglitazone lowers blood pressure and enhances insulin sensitivity in Watanabe heritable hyperlipidemic rabbits. *Am. J. Hypertens.* **1997**, *10*, 1027–1033.

(269) Kemnitz, J. W.; Elson, D. F.; Roecker, E. B.; Baum, S. T.; Bergman, R. N.; Meglasson, M. D. Pioglitazone increases insulin sensitivity, reduces blood glucose, insulin, and lipid levels, and lowers blood pressure in obese, insulin resistant rhesus monkeys. *Diabetes* **1994**, *43*, 204–211.

(270) Zhang, H. Y.; Reddy, S. R.; Kotchen, T. A. Antihypertensive effect of pioglitazone is not invariably associated with increased insulin sensitivity. *Hypertension* **1994**, *24*, 106–110.

(271) Kotchen, T. A. Attenuation of hypertension by insulin-sensitizing agents. *Hypertension* **1996**, *28*, 219–223.

(272) Morikang, E.; Benson, S. C.; Kurtz, T. W.; Pershadsingh, H. A. Effects of thiazolidinediones on growth and differentiation of human aorta and coronary myocytes. *Am. J. Hypertens.* **1997**, *10*, 440–446.

(273) Law, R. E.; Meehan, W. P.; Xi, X.-P.; Graf, K.; Wuthrich, D. A.; Coats, W.; Faxon, D.; Hsueh, W. A. Troglitazone inhibits vascular smooth muscle cell growth and intimal hyperplasia. *J. Clin. Invest.* **1996**, *98*, 1897–1905.

(274) Yoshimoto, T.; Naruse, M.; Shizume, H.; Naruse, K.; Tanabe, A.; Tanaka, M.; Tago, K.; Irie, K.; Muraki, T.; Demura, H.; Zardi, L. Vasculo-protective effects of insulin sensitizing agent pioglitazone in neointimal thickening and hypertensive vascular hypertrophy. *Atherosclerosis* **1999**, *145*, 333–340.

(275) Graf, K.; Xi, X.-P.; Hsueh, W. A.; Law, R. E. Troglitazone inhibits angiotensin II-induced DNA synthesis and migration in vascular smooth muscle cells. *FEBS Lett.* **1997**, *400*, 119–121.

(276) Force, T.; Bonventre, J. V. Growth factors and mitogen-activated protein kinases. *Hypertension* **1998**, *31*, 152–161.

(277) Kotchen, T. A.; Zhang, H. Y.; Reddy, S.; Hoffmann, R. G. Effect of pioglitazone on vascular reactivity in vivo and in vitro. *Am. J. Physiol.* **1996**, *270*, R660–R666.

(278) Itoh, H.; Doi, K.; Tanaka, T.; Fukunaga, Y.; Hosoda, K.; Inoue, G.; Nishimura, H.; Yoshimasa, Y.; Yamori, Y.; Nakao, K. Hypertension and insulin resistance: role of peroxisome proliferator-activated receptor γ. *Clin. Exp. Pharmacol. Physiol.* **1999**, *26*, 558–560.

(279) Satoh, H.; Tsukamoto, K.; Hashimoto, Y.; Hashimoto, N.; Togo, M.; Hara, M.; Maekawa, H.; Isoo, N.; Kimura, S.; Watanabe, T. Thiazolidinediones suppress endothelin-1 secretion from bovine vascular endothelial cells: a new possible role of PPARγ on vascular endothelial function. *Biochem. Biophys. Res. Commun.* **1999**, *254*, 757–763.

(280) Kato, K.; Satoh, H.; Endo, Y.; Yamada, D.; Midorikawa, S.; Sato, W.; Mizuno, K.; Fujita, T.; Tsukamoto, K.; Watanabe, T. Thiazolidinediones down-regulate plasminogen activator inhibitor type 1 expression in human vascular endothelial cells: a possible role for PPARγ in endothelial function. *Biochem. Biophys. Res. Commun.* **1999**, *258*, 431–435.

(281) Fonseca, V. A.; Reynolds, T.; Hemphill, D.; Randolph, C.; Wall, J.; Valiquet, T. R.; Graveline, J.; Fink, L. M. Effect of troglitazone on fibrinolysis and activated coagulation in patients with non-insulin-dependent diabetes mellitus. *J. Diabetes Complications* **1998**, *12*, 181–186.

(282) Zhang, F.; Sowers, J. R.; Ram, J. L.; Standley, P. R.; Peuler, J. D. Effects of pioglitazone on calcium channels in vascular smooth muscle. *Hypertension* **1994**, *24*, 170–175.

(283) Knock, G. A.; Mishra, S. K.; Aaronson, P. I. Differential effects of insulin-sensitizers troglitazone and rosiglitazone on ion currents in rat vascular myocytes. *Eur. J. Pharmacol.* **1999**, *368*, 103–109.

(284) Souza, S. C.; Yamamoto, M. T.; Franciosa, M. D.; Lien, P.; Greenberg, A. S. BRL 49653 blocks the lipolytic actions of tumor necrosis factor-α: A potential new insulin-sensitizing mechanism for thiazolidinediones. *Diabetes* **1998**, *47*, 691–695.

(285) Chinetti, G.; Griglio, S.; Antonucci, M.; Torra, I. P.; Delerive, P.; Majd, Z.; Fruchart, J.-C.; Chapman, J.; Najib, J.; Staels, B. Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages. *J. Biol. Chem.* **1998**, *273*, 25573–25580.

(286) Marx, N.; Sukhova, G.; Murphy, C.; Libby, P.; Plutzky, J. Macrophages in human atheroma contain PPARγ: differentiation-dependent peroxisomal proliferator-activated receptor γ (PPARγ) expression and reduction of MMP-9 activity through PPARγ activation in mononuclear phagocytes in vitro. *Am. J. Pathol.* **1998**, *153*, 17–23.

(287) Ricote, M.; Li, A. C.; Willson, T. M.; Kelly, C. J.; Glass, C. K. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* **1998**, *391*, 79–82.

(288) Jiang, C.; Ting, A. T.; Seed, B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998, *391*, 82–86.

(289) Su, C. G.; Wen, X.; Bailey, S. T.; Jiang, W.; Rangwala, S. M.; Keilbaugh, S. A.; Flanigan, A.; Murthy, S.; Lazar, M. A.; Wu, G. D. A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response. *J. Clin. Invest.* 1999, *104*, 383–389.

(290) Steinberg, D. Low-density lipoprotein oxidation and its pathobiological significance. *J. Biol. Chem.* 1997, *272*, 20963–20966.

(291) Spiegelman, B. M. PPARγ in monocytes: less pain, any gain? *Cell* 1998, *93*, 153–155.

(292) Ehrmann, D. A.; Schneider, D. J.; Sobel, B. E.; Cavaghan, M. K.; Imperial, J.; Rosenfield, R. L.; Polonsky, K. S. Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 1997, *82*, 2108–2116.

(293) Minamikawa, J.; Tanaka, S.; Yamauchi, M.; Inoue, D.; Koshiyama, H. Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes. *J. Clin. Endocrinol. Metab.* 1998, *83*, 1818–1820.

(294) Altiok, S.; Xu, M.; Spiegelman, M. PPARγ induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. *Genes Dev.* 1997, *11*, 1987–1998.

(295) Tontonoz, P.; Singer, S.; Forman, B. M.; Sarraf, P.; Fletcher, J. A.; Fletcher, C. D. M.; Brun, R. P.; Mueller, E.; Altiok, S.; Oppenheim, H.; Evans, R. M.; Spiegelman, B. M. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor. *Proc. Natl. Acad. Sci. U.S.A.* 1997, *94*, 237–241.

(296) Demetri, G. D.; Fletcher, C. D. M.; Mueller, E.; Sarraf, P.; Naujoks, R.; Campbell, N.; Spiegelman, B. M.; Singer, S. Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-γ ligand troglitazone in patients with liposarcoma. *Proc. Natl. Acad. Sci. U.S.A.* 1999, *96*, 3951–3956.

(297) Mueller, E.; Sarraf, P.; Tontonoz, P.; Evans, R. M.; Martin, K. J.; Zhang, M.; Fletcher, C.; Singer, S.; Spiegelman, B. M. Terminal differentiation of human breast cancer through PPARγ. *Mol. Cell* 1998, *1*, 465–470.

(298) Elstner, E.; Muller, C.; Koshizuka, K.; Williamson, E. A.; Park, D.; Asou, H.; Shintaku, P.; Said, J. W.; Heber, D.; Koeffler, H. P. Ligands for peroxisome proliferator-activated receptor γ and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells in vitro and in BNX mice. *Proc. Natl. Acad. Sci. U.S.A.* 1998, *95*, 8806–8811.

(299) Suh, N.; Wang, Y.; Williams, C. R.; Risingsong, R.; Gilmer, T.; Willson, T. M.; Sporn, M. B. A new ligand for the peroxisome proliferator activated receptor-γ (PPAR-γ), GW7845, inhibits rat mammary carcinogenesis. *Cancer Res.* 1999, *59*, 5671–5673.

(300) Gimble, J. M.; Pighetti, G. M.; Lerner, M. R.; Wu, X.; Lightfoot, S. A.; Brackett, D. J.; Darcy, K.; Hollingsworth, A. B. Expression of peroxisome proliferator activated receptor mRNA in normal and tumorigenic rodent mammary glands. *Biochem. Biophys. Res. Commun.* 1998, *253*, 813–817.

(301) Sarraf, P.; Mueller, E.; Jones, D.; King, F. J.; DeAngelo, D. J.; Partridge, J. B.; Holden, S. A.; Chen, L. B.; Singer, S.; Fletcher, C.; Spiegelman, B. M. Differentiation and reversal of malignant changes in colon cancer through PPARγ. *Nat. Med.* 1998, *4*, 1046–1052.

(302) Brockman, J. A.; Gupta, R. A.; DuBois, R. N. Activation of PPARγ leads to inhibition of anchorage-independent growth of human colorectal cancer cells. *Gastroenterology* 1998, *115*, 1049–1055.

(303) Sarraf, P.; Mueller, E.; Smith, W. M.; Wright, H. M.; Kum, J. B.; Aaltonen, L. A.; De la Chapelle, A.; Spiegelman, B. M.; Eng, C. Loss-of-function mutations in PPARγ associated with human colon cancer. *Mol. Cell* 1999, *3*, 799–804.

(304) Lefebvre, A.-M.; Chen, I.; Desreumaux, P.; Nahib, J.; Fruchart, J.-C.; Geboes, K.; Briggs, M.; Heyman, R.; Auwerx, J. Activation of the peroxisome proliferator-activated receptor γ promotes the development of colon tumors in C57BL/6J-APC^Min/+ mice. *Nat. Med.* 1998, *4*, 1053–1057.

(305) Saez, E.; Tontonoz, P.; Nelson, M. C.; Alvarez, J. G. A.; U, T. M.; Baird, S. M.; Thomazy, V. A.; Evans, R. M. Activators of the nuclear receptor PPARγ enhance colon polyp formation. *Nat. Med.* 1998, *4*, 1058–1061.

(306) Schmidt, A.; Endo, N.; Rutledge, S. J.; Vogel, R.; Shinar, D.; Rodan, G. A. Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. *Mol. Endocrinol.* 1992, *6*, 1634–1641.

(307) Chen, F.; Law, S. W.; O'Malley, B. W. Identification of two mPPAR related receptors and evidence for the existence of five subfamily members. *Biochem. Biophys. Res. Commun.* 1993, *196*, 671–677.

(308) Amri, E.-Z.; Bonino, F.; Ailhaud, G.; Abumrad, N. A.; Grimaldi, P. A. Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J. Biol. Chem.* 1995, *270*, 2367–2371.

(309) Johnson, T. E.; Holloway, M. K.; Vogel, R.; Rutledge, S. J.; Perkins, J. J.; Rodan, G. A.; Schmidt, A. Structural requirements and cell-type specificity for ligand activation of peroxisome proliferator-activated receptors. *J. Steroid Biochem. Mol. Biol.* 1997, *63*, 1–8.

(310) Brooks, C. D. W.; Summers, J. B. Modulators of leukotriene biosynthesis and receptor activation. *J. Med. Chem.* 1996, *39*, 2629–2654.

(311) Schmidt, A.; Vogel, R. L.; Witherup, K. M.; Rutledge, S. J.; Pitzenberger, S. M.; Adam, M.; Rodan, G. A. Identification of fatty acid methyl ester as naturally occurring transcriptional regulators of the members of the peroxisome proliferator-activated receptor family. *Lipids* 1996, *31*, 1115–1124.

(312) Bastie, C.; Holst, D.; Gaillard, D.; Jehl-Pietri, C.; Grimaldi, P. A. Expression of peroxisome proliferator-activated receptor PPARδ promotes induction of PPARγ and adipocyte differentiation in 3T3C2 fibroblasts. *J. Biol. Chem.* 1999, *274*, 21920–21925.

(313) Brun, R. P.; Tontonoz, P.; Forman, B. M.; Ellis, R.; Chen, J.; Evans, R. M.; Spiegelman, B. M. Differential activation of adipogenesis by multiple PPAR isoforms. *Genes Dev.* 1996, *10*, 974–984.

(314) Lim, H.; Gupta, R. A.; Ma, W.-G.; Paria, B. C.; Moller, D. E.; Morrow, J. D.; DuBois, R. N.; Trzaskos, J. M.; Dey, S. K. Cyclooxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ. *Genes Dev.* 1999, *13*, 1561–1574.

(315) He, T.-C.; Chan, T. A.; Vogelstein, B.; Kinzler, K. W. PPARδ is an APC-regulated target of nonsteroidal antiinflammatory drugs. *Cell* 1999, *99*, 335–345.

(316) Auwerx, J. Regulation of gene expression by fatty acids and fibric acid derivatives: An integrative role for peroxisome proliferator activated receptors. *Horm. Res.* 1992, *38*, 269–277.

(317) Desvergne, B.; Ijpenberg, A.; Devchand, P. R.; Wahli, W. The peroxisome proliferator-activated receptors at the crossroad of diet and hormonal signaling. *J. Steroid Biochem. Mol. Biol.* 1998, *65*, 65–74.

(318) Cobb, J.; Dukes, I. Recent advances in the development of agents for the treatment of type 2 diabetes. *Annu. Rep. Med. Chem.* 1998, *33*, 213–222.

(319) Sliskovic, D. R.; Krause, B. R.; Bocan, T. M. A. Atherosclerosis: emerging pharmacological approaches. *Annu. Rep. Med. Chem.* 1999, *34*, 101–110.

(320) Glaxo Wellcome development pipeline at http://www.glaxowellcome.com/pipeline.htm.

(321) Grundy, S. M. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am. J. Cardiol.* 1999, *83*, 25F–29F.

(322) Ziboh, V. A. The significance of polyunsaturated fatty acids in cutaneous biology. *Lipids* 1996, *31*, S249–S253.

(323) Belch, J. J. F.; Muir, A. n-6 and n-3 essential fatty acids in rheumatoid arthritis and other rheumatic conditions. *Proc. Nutr. Soc.* 1998, *57*, 563–569.

(324) Kolonel, L. N.; Nomura, A. M. Y.; Cooney, R. V. Dietary fat and prostate cancer: current status. *J. Natl. Cancer Inst.* 1999, *91*, 414–428.

(325) Woutersen, R. A.; Appel, M. J.; Van Garderen-Hoetmer, A.; Wijnands, M. V. W. Dietary fat and carcinogenesis. *Mutat. Res.* 1999, *443*, 111–127.

(326) DeRisi, J. L.; Iyer, V. R.; Brown, P. O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 1997, *278*, 680–686.

JM990554G

Review

Physiological and Therapeutic Roles of Peroxisome Proliferator-Activated Receptors

JOEL BERGER, Ph.D.,[1] and JOHN A. WAGNER, M.D., Ph.D.[2]

ABSTRACT

The peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor isoforms, including PPARγ, PPARα, and PPARδ, encoded by different genes. PPARs are ligand-regulated transcription factors that control gene expression by binding to specific response elements (PPREs) within promoters. PPARs bind as heterodimers with a retinoid X receptor and, upon binding agonist, interact with cofactors increasing the rate of transcription initiation. The PPARs play a critical physiological role as lipid sensors and regulators of lipid metabolism. Natural ligands for the PPARs include fatty acids and eicosanoids. More potent synthetic PPAR ligands, including the fibrates and thiazolidinediones, are effective in the treatment of dyslipidemia and diabetes. Use of selective ligands led to the discovery of additional potential roles for the PPARs in pathological states, including atherosclerosis, inflammation, and hypertension. This review provides an overview of the molecular mechanisms of PPAR action and the involvement of the PPARs in the etiology and treatment of several chronic diseases.

INTRODUCTION

THE PEROXISOME PROLIFERATOR-ACTIVATED receptors (PPARs) form a subfamily in the nuclear receptor superfamily. Three isoforms, encoded by separate genes, have been identified thus far: PPARγ, PPARα, and PPARδ.

There are two PPARγ isoforms expressed at the protein level in mouse[1] and human,[2] γ1 and γ2. They differ only in that the latter has 30 additional amino acids at its N terminus due to differential promoter usage within the same gene, and subsequent alternative RNA processing. PPARγ2 is expressed primarily in adipose tissue,[3] while PPARγ1 is expressed in a broad range of tissues.

Murine PPARα was the first member of this nuclear receptor subclass to be cloned[4]; it has since been cloned from humans.[5] PPARα is expressed in numerous metabolically active tissues, including liver, kidney, heart, skeletal muscle, and brown fat.[6,7] It is also present in monocytes,[8] vascular endothelium,[9] and vascular smooth muscle cells.[10] Activation of PPARα induces hepatic peroxisome proliferation, hepatomegaly, and hepatocarcinogenesis in rodents.[4] These toxic effects are lost in humans, although the same compounds activate PPARα across species.[11]

Departments of [1]Metabolic Disorders–Diabetes and [2]Clinical Pharmacology, Merck Research Laboratories, Rahway, New Jersey.

Human PPARδ was cloned in the early 1990s[12] and subsequently cloned from rodents.[13,14] PPARδ is expressed in a wide range of tissues and cells with the highest levels of expression found in digestive tract, heart, kidney, liver, adipose, and brain.[15] Thus far, no PPARδ-specific gene targets have been identified.

As shown in Figure 1, the PPARs are ligand-dependent transcription factors that regulate target gene expression by binding to specific peroxisome proliferator response elements (PPREs) in enhancer sites of regulated genes. PPARs possess a modular structure composed of functional domains[16] that include a DNA binding domain (DBD) and a ligand binding domain (LBD). The DBD specifically binds PPREs in the regulatory region of PPAR-responsive genes. The DBD, located in the C-terminal half of the receptor contains the ligand-dependent activation domain, AF-2. Each receptor binds to its PPRE as a heterodimer with a retinoid X receptor (RXR). Upon binding an agonist, the conformation of a PPAR is altered and stabilized such that a binding cleft, made up in part of the AF-2 domain, is created and recruitment of transcriptional coactivators occurs. Coactivators augment the ability of nuclear receptors to initiate the transcription process.[17,18] The result of the agonist-induced PPAR-coactivator interaction at the PPRE is an increase in gene transcription. Downregulation of gene expression by PPARs appears, thus far, to occur through indirect mechanisms.[19,20]

The first cloning of a PPAR (PPARα) occurred in the course of the search for the molecular target of rodent hepatic peroxisome proliferating agents. Since then, numerous fatty acids and their derivatives including a variety of eicosanoids and prostaglandins have been shown to serve as ligands of the PPARs. Thus, these receptors may play a central role in the sensing of nutrient levels and in the modulation of their metabolism. In addition, PPARs are the primary targets of selected classes of synthetic compounds that have been used in the successful treatment of diabetes and dyslipidemia. As such, an understanding of the molecular and physiological characteristics of these receptors has become extremely important to the development and utilization of drugs used to treat metabolic disorders. In addition, due to the great interest within the research community, a wide range of additional, putative roles for the PPARs have been discovered; PPARα and PPARγ may play a role in a wide range of events involving the vasculature, including atherosclerotic plaque formation and stability, thrombosis, vascular tone, and angiogenesis. New therapeutic uses for PPAR ligands may arise from these discoveries.

SYNTHETIC LIGANDS

Ligands for PPARs are summarized in Table 1. Thiazolidinediones (TZDs) were originally developed on the basis of their insulin-sensitizing effects in animal pharmacology studies. Subsequently, it was found that TZDs induced adipocyte differentiation and increased expression of adipocyte genes, including the adipocyte fatty acid–binding protein aP2.[21-23] Independently, it was discovered that PPARγ interacted with a regulatory element of the aP2 gene that controlled its adipocyte-specific expression.[24] On the basis of these seminal observations, experiments were performed that determined that TZDs were PPARγ ligands and agonists and demonstrate a definite correlation between their in vitro PPARγ activities and their in vivo insulin-sensitizing actions.[25-27]

Several TZDs, including troglitazone, rosiglitazone, and pioglitazone, have insulin-sensitizing and anti-diabetic activity in humans with type 2 diabetes and impaired glucose tolerance.[28,29] Farglitazar is a very potent non-TZD PPARγ-selective agonist that was recently shown to have antidiabetic as well as lipid-altering efficacy in humans.[29] In addition to these potent PPARγ ligands, a subset of the nonsteroidal antiinflammatory drugs (NSAIDs), including indomethacin, fenoprofen, and ibuprofen, have displayed weak PPARγ and PPARα activities.[31]

The fibrates, amphipathic carboxylic acids that have been proven useful in the treatment of hypertriglyceridemia, are PPARα ligands. The prototypical member of this compound class, clofibrate, was developed prior to the identification of PPARs, using in vivo assays in rodents to assess lipid-lowering efficacy.[32]
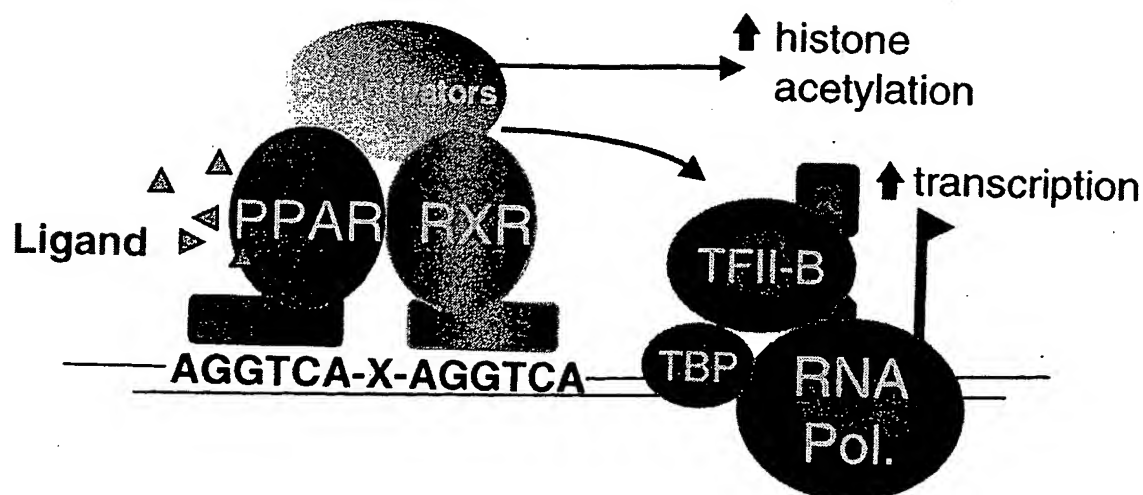
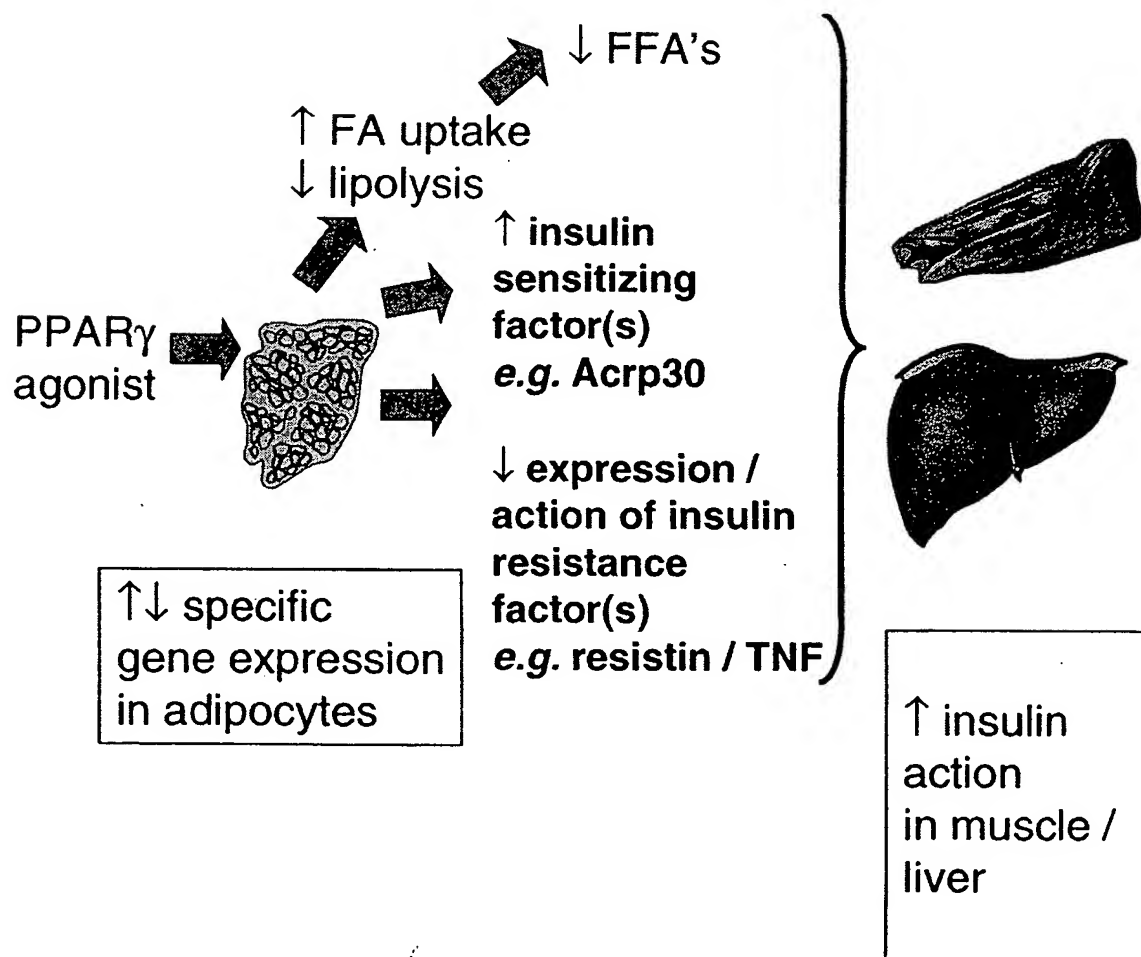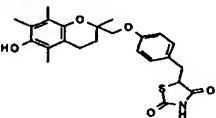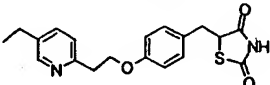FIG. 1. Mechanism of transcriptional activation by PPAR isoforms.

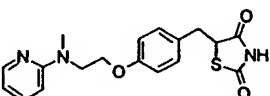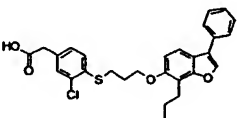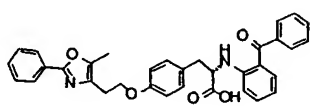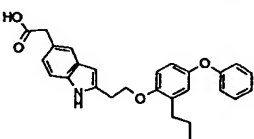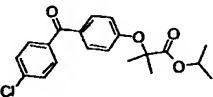FIG. 2. Working hypothesis for PPARγ-mediated increases in insulin sensitivity.

TABLE 1. SELECTED SYNTHETIC PPAR LIGANDS

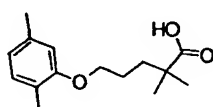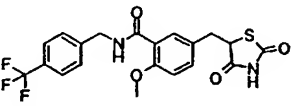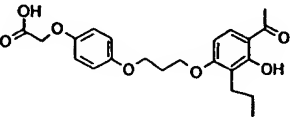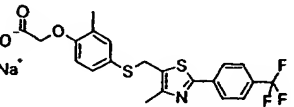|	Chemical structure	PPARγ	PPARα	PPARδ
Troglitazone		5,000 nM	>10 μM	>30 μM
Pioglitazone		3,000 nM	20–40 μM	>30 μM
Rosiglitazone		200 nM	>20 μM	>30 μM
L-796449		20 nM	5 nM	30 nM
Farglitazar		20 nM	1,000 nM	>5 μM
L-805645		50 nM	2,000 nM	>10 μM
Fenofibrate		>30 μM	20 μM	>40 μM
Gemfibrozil		>50 μM	40 μM	>50 μM
KRP-297		1,000 nM	1,500 nM	>10 μM
L-165041		>5,000 nM	50 nM	>10 μM
GW-501516		>1,000 nM	>1,000 nM	1 nM

Compounds with binding activity (IC_{50} or Ki values) to recombinant human PPAR isoforms.

Clofibrate and fenofibrate have been shown to activate PPARα with a 10-fold selectivity over PPARγ.[29] Bezafibrate acted as a pan-agonist that showed similar potency on all three PPAR isoforms. Wy-14643, the 2-arylthioacetic acid analogue of clofibrate, was a potent murine PPARα agonist as well as a weak PPARγ agonist. In humans, all of the fibrates must be used at high doses (200–1,200 mg/day) to achieve efficacious lipid-lowering activity.

TZDs and non-TZDs have also been identified that are dual PPARγ/α agonists. By virtue

of the additional PPARα agonist activity, this class of compounds has potent lipid-altering efficacy in addition to antihyperglycemic activity in animal models of diabetes and lipid disorders. KRP-297 is an example of a TZD dual PPARγ/α agonist[3]; DRF-2725[33] and AZ-242[34] are non-TZD dual PPARγ/α agonists.

In order to define the physiological role of PPARδ, efforts have been made to develop novel compounds that activate this receptor in a selective manner. Amongst the α-substituted carboxylic acids we previously described,[35] the potent PPARδ ligand L-165041 demonstrated approximately 30-fold agonist selectivity for this receptor over PPARγ; it was inactive on murine PPARα. This compound was found to increase high-density lipoprotein levels in rodents.[36] Recently, Oliver et al.[37] reported that GW501516 was a potent, highly-selective PPARδ agonist that produced beneficial changes in serum lipid parameters in obese, insulin-resistant rhesus monkeys.

INSULIN RESISTANCE AND DIABETES

PPARγ is necessary and sufficient for the differentiation of adipocytes *in vitro* and *in vivo*.[38–41] In adipocytes, PPARγ increases the expression of numerous genes involved in lipid metabolism and lipid uptake.[42–47] In contrast, PPARγ down-regulates leptin, a secreted, adipocyte-selective protein that has been shown to inhibit feeding and augment catabolic lipid metabolism.[48,49] This receptor activity might explain the increased caloric uptake and storage noted *in vivo* upon treatment with PPARγ agonists. Clinically, TZDs, including troglitazone, rosiglitazone, and pioglitazone,[28,29] and non-TZDs, including farglitazar,[29] have insulin-sensitizing and antidiabetic activity.

PPARγ has been associated with several genes that affect insulin action. TNFα, a proinflammatory cytokine that is expressed by adipocytes, has been associated with insulin resistance.[50] PPARγ agonists inhibited expression of TNFα in adipose tissue of obese rodents,[51] and ablated the actions of TNFα in adipocytes *in vitro*.[52] We demonstrated that PPARγ agonists inhibit expression of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD-1), the enzyme that converts cortisone to the glucocorticoid agonist cortisol, in adipocytes and adipose tissue of type 2 diabetes mouse models.[20] This is noteworthy since hypercorticosteroidism exacerbates insulin resistance.[53,54] Adipocyte Complement-Related Protein of 30 kDa (Acrp30 or adiponectin) is a secreted adipocyte-specific protein that decreases glucose, triglycerides, and free fatty acids.[55,56] In comparison to normal human subjects, patients with type 2 diabetes have reduced plasma levels of Acrp30.[57] We found that treatment of diabetic mice and nondiabetic human subjects with PPARγ agonists increased plasma levels of Acrp30.[58] Induction of Acrp30 by PPARγ agonists might therefore also play a key role in the insulin-sensitizing mechanism of PPARγ agonists in diabetes.

PPARγ is expressed predominantly in adipose tissue. Thus, the prevailing hypothesis regarding the net *in vivo* efficacy of PPARγ agonists involves direct actions on adipose cells with secondary effects in key insulin responsive tissues such as skeletal muscle and liver. This hypothesis is supported by the lack of glucose-lowering efficacy of rosiglitazone in a mouse model of severe insulin resistance where white adipose tissue was essentially absent.[59] Furthermore, we have shown that *in vivo* treatment of insulin resistant rats produces acute (<24 h) normalization of adipose tissue insulin action whereas insulin-mediated glucose uptake in muscle was not improved until several days after the initiation of therapy.[60] This is consistent with the fact that PPARγ agonists can produce an increase in adipose tissue insulin action after direct *in vitro* incubation,[61] whereas no such effect could be demonstrated using isolated *in vitro* incubated skeletal muscles.[60] The beneficial metabolic effects of PPARγ agonists on muscle and liver may be mediated by their ability to (a) enhance insulin-mediated adipose tissue uptake, storage (and potentially catabolism) of free fatty acids[62]; (b) induce the production of adipose-derived factors with potential insulin sensitizing activity (e.g., Acrp30); and/or (c) suppress the circulating levels and/or actions of insulin resistance–causing adipose-derived factors

such as TNFα or resistin.[63] These steps are summarized in Figure 2.

DYSLIPIDEMIA AND ATHEROSCLEROSIS

PPARα has been shown to play a critical role in the regulation of cellular uptake, activation, and β-oxidation of fatty acids. Activation of PPARα induces expression of fatty acid transport proteins and enzymes in the peroxisomal β-oxidation pathway.[44,46,64–68] Several mitochondrial enzymes involved in the energy-harvesting catabolism of fatty acids are robustly upregulated by PPARα agonists. Peroxisome proliferators also activate expression of the CYP4As, a subclass of cytochrome P450 enzymes that catalyze the ω-hydroxylation of fatty acids, a pathway that is particularly active in the fasted and diabetic states. In sum, it is clear that PPARα is an important lipid sensor and regulator of cellular energy-harvesting metabolism.

Atherosclerosis is a very prevalent disease in Westernized societies. In addition to a strong association with elevated LDL cholesterol, "dyslipidemia" characterized by elevated triglyceride-rich particles and low levels of HDL cholesterol is commonly associated with other aspects of a metabolic syndrome that includes obesity, insulin resistance, type 2 diabetes, and an increased risk of coronary artery disease.[69] Thus, in 8,500 men with known coronary artery disease, 38% were found to have low HDL (<35 mg/dL) and 33% had elevated triglycerides (>200 mg/dL).[70] In such patients, treatment with fibrates resulted in substantial triglyceride lowering and modest HDL-raising efficacy.[71] More importantly, a recent large prospective trial showed that treatment with gemfibrozil produced a 22% reduction in cardiovascular events or death.[71,72] Thus PPARα agonists can effectively improve cardiovascular risk factors and have a net benefit to improve cardiovascular outcomes. In fact, fenofibrate was recently approved in the United States for treatment of type IIA and IIB hyperlipidemia. Mechanisms by which PPARα activation cause triglyceride lowering are likely to include the effects of agonists to suppress hepatic apo-CIII gene expression while also stimulating lipoprotein lipase gene expression.[45,73]

Dual PPARγ/α agonists, including KRP-297[30] and DRF 2725,[33] possess potent lipid-altering efficacy in addition to antihyperglycemic activity in animal models of diabetes and lipid disorders.

The presence of PPARα and/or PPARγ expression in vascular cell types, including macrophages, endothelial cells, and vascular smooth muscle cells, suggests that direct vascular effects might contribute to potential antiatherosclerosis efficacy.[69] PPARα[74] and PPARγ[75–77] activation have been shown to inhibit cytokine-induced vascular cell adhesion and to suppress monocyte-macrophage migration. Several additional studies have also shown that PPARγ-selective compounds have the capacity to reduce arterial lesion size and attenuate monocyte-macrophage homing to arterial lesions in animal models of atherosclerosis.[77–81] In addition, two recent studies have suggested that either PPARα[82] or PPARγ[82,83] activation in macrophages can induce the expression of a cholesterol efflux "pump" protein.

We have determined that relatively selective PPARδ agonists produce minimal, if any, glucose- or triglyceride-lowering activity in murine models of type 2 diabetes in comparison with efficacious PPARγ or PPARα agonists.[35] Subsequently, a modest increase in HDL-cholesterol levels was detected with PPARδ agonists in db/db mice.[36] Recently, Oliver et al.[37] reported that a potent, selective PPARδ agonist could induce a substantial increase in HDL-cholesterol levels while reducing triglyceride levels and insulin resistance in obese rhesus monkeys.[37]

Thus, via multifactorial mechanisms that include improvements in circulating lipids, systemic and local antiinflammatory effects, and, potentially, inhibition of vascular cell proliferation, PPARα, PPARγ, and, perhaps, PPARδ agonists have a strong potential use in the treatment or prevention of atherosclerosis.

INFLAMMATION

Monocytes and macrophages are known to play an important part in the inflammatory

process through the release of inflammatory cytokines and the production of nitric oxide by inducible nitric oxide synthase. It has been demonstrated that rosiglitazone induced apoptosis of macrophages at concentrations that paralleled its affinity for PPARγ.[8] This ligand has also been show to block inflammatory cytokine synthesis in colonic cell lines.[84] This latter observation offers a possible mechanistic explanation for the observed antiinflammatory actions of TZDs in rodent models of colitis.[84,85]

Antiinflammatory actions have been described for PPARα ligands that may be important in the maintenance of vascular health. Treatment of cytokine-activated human macrophages with PPARα agonists induced apoptosis of the cells.[8] Staels et al.[10] reported that PPARα agonists inhibited activation of aortic smooth muscle cells in response to inflammatory stimuli. In hyperlipidemic patients, fenofibrate treatment decreased the plasma concentrations of the inflammatory cytokine interleukin-6.[10] Additional research should further investigate these provocative results and deepen our knowledge of a role for PPARα in the physiopathology of the inflammatory process within the vasculature.

HYPERTENSION

Hypertension is a complex disorder of the cardiovascular system that has been shown to be associated with insulin resistance. Type 2 diabetes patients demonstrate a 1.5–2-fold increase in hypertension in comparison with the general population.[86,87] Troglitazone, rosiglitazone, and pioglitazone therapy have been shown to decrease blood pressure in diabetic patients[88–91] as well as troglitazone therapy in obese, insulin-resistant persons.[92,93] Since such reductions in blood pressure were shown to correlate with decreases in insulin levels,[89] they may be mediated—at least in part—by an improvement in insulin sensitivity. However, since TZDs also lowered blood pressure in one-kidney one-clip Sprague Dawley rats,[94] which are not insulin resistant, it was proposed that the hypotensive action of PPARγ agonists is not exerted solely through their ability to improve insulin sensitivity. Other mechanisms that have

been invoked to explain the antihypertensive effects of PPARγ agonists include their ability to (a) downregulate expression of peptides that control vascular tone such as PAI-1,[95,96] endothelin,[97,98] and type-c natriuretic peptide C[97]; or (b) alter calcium concentrations and the calcium sensitivity of vascular cells.[99,100] Additional research will be required in order to determine the primary mechanisms by which PPARγ ligands lower vascular resistance.

CONCLUSION

Isoforms of the PPAR family of nuclear receptors are clearly involved in the systemic regulation of lipid metabolism and serve as "sensors" for fatty acids, prostanoid metabolites, eicosanoids and related molecules. These receptors function to regulate a broad array of genes in a coordinate fashion. Important biochemical pathways that regulate insulin action, lipid oxidation, lipid synthesis, adipocyte differentiation, peroxisome function, cell apoptosis, and inflammation can be modulated through the individual PPAR isoforms. Strong therapeutic effects of PPARα and PPARγ agonists to favorably influence systemic lipid levels, glucose homeostasis, and atherosclerosis risk (in the case of PPARα activation in humans) have recently been discovered. PPARα and PPARγ agonists are presently used clinically to favorably alter systemic lipid levels and glucose homeostasis, respectively. Recent observations made using PPARδ ligands suggest that this less well-characterized isoform may also be an important therapeutic target for dyslipidemia and insulin resistance, as well. Further assessment of the molecular and physiologic effects of individual PPARs will lead to a more complete understanding of the potential utilities as well as toxicities of pharmacologically modulating PPAR activities.

REFERENCES

1. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Sambasiva Rao M, Reddy JK: Structural organization of mouse peroxisome proliferator-activated receptor γ (mPPARγ) gene: alternative promoter use and different splicing yield two mPPARg isoforms. Proc Natl Acad Sci USA 1995;92:7921–7925.

2. Elbrecht A, Chen Y, Cullinan CA, Hayes N, Leibowitz MD, Moller DE, Berger J: Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma1 and gamma2. Biochem Biophys Res Comm 1996;224: 431–437.

3. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J: The organization, promoter analysis, and expression of the human PPARgamma gene. J Biol Chem 1997;272:18779–18789.

4. Issemann I, Green S: Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 1990;347:645–649.

5. Sher T, Yi H-F, McBride OW, Gonzalez FJ: cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. Biochemistry 1993;32:5598–5604.

6. Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W: Differential expression of peroxisome proliferator-activated receptor (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology 1996;137:354–366.

7. Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M, Vidal H: Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor$_\alpha$ in humans. Diabetes 1997;46:1319–1327.

8. Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, Fruchart JC, Chapman J, Najib J, Staels B: Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. J Biol Chem 1998;273:25573–25580.

9. Inoue I, Shino K, Noji S, Awata T, Katayama S: Expression of peroxisome proliferator-activated receptor alpha (PPAR$_{alpha}$) in primary cultures of human vascular endothelial cells. Biochem Biophys Res Commun 1998;246:370–374.

10. Staels B, Koenig W, Habib A, Merval R, Lebret M, Torra IP, Delerive P, Fadel A, Chinetti G, Fruchart JC, Najib J, Maclouf J, Tedgui A: Activation of human aortic smooth-muscle cells is inhibited by PPAR$_{alpha}$ but not by PPAR$_{gamma}$ activators. Nature 1998;393:790–793.

11. Cattley RC, DeLuca J, Elcombe C, Fenner-Crisp P, Lake BG, Marsman DS, Pastoor TA, Popp JA, Robinson DE, Schwetz B, Tugwood J, Wahli W: Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? Regul Toxicol Pharmacol 1998;27:47–60.

12. Schmidt A, Endo N, Rutledge SJ, Vogel R, Shinar D, Rodan GA: Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acid. Mol Endocrinol 1992;6:1634–1641.

13. Kliewer SA, Forman BM, Blumberg B, Ong ES,

Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM: Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci USA 1994;91:7355–7359.

14. Mukherjee R, Jow L, Noonan D, McDonnell DP: Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. J Steroid Biochem Mol Biol 1994;51:157–166.

15. Escher P, Braissant O, Basu-Modak S, Michalik L, Wahli W, Desvergne B: Rat PPARs: quantitative analysis in adult rat tissues and regulation in fasting and refeeding. Endocrinology 2001;142:4195–4202.

16. Laudet V, Hanni C, Coll J, Catzeflis F, Stehelin D: Evolution of the nuclear receptor gene superfamily. EMBO J 1992;11:1003–1013.

17. Xu L, Glass CK, Rosenfeld MG: Coactivator and corepressor complexes in nuclear receptor function. Curr Opin Genet Dev 1999;9:140–147.

18. McKenna NJ, O'Malley BW: From ligand to response: generating diversity in nuclear receptor coregulator function. J Steroid Biochem Mol Biol 2000;74:351–356.

19. Hollenberg AN, Susulic VS, Madura JP, Zhang B, Moller DE, Tontonoz P, Sarraf P, Spiegelman BM, Lowell BB: Functional antagonism between CCAAT/enhancer binding protein-alpha and peroxisome proliferator-activated receptor-gamma on the leptin promoter. J Biol Chem 1997;272:5283–5290.

20. Berger J, Tanen M, Elbrecht A, Hermanowski-Vosatka A, Moller DE, Wright SD, Thieringer R: Peroxisome proliferator-activated receptor-gamma ligands inhibit adipocyte 11$_{beta}$-hydroxysteroid dehydrogenase type 1 expression and activity. J Biol Chem 2001;276:12629–12635.

21. Kletzien RF, Clarke SD, Ulrich RG: Enhancement of adipocyte differentiation by an insulin-sensitizing agent. Mol Pharmacol 1992;41:393–398.

22. Ibrahimi A, Teboul L, Gaillard D, Amri E, Ailhaud G, Young P, Cawthorne M, Grimaldi P: Evidence for a common mechanism of action for fatty acids and thiazolidinedione antidiabetic agents on gene expression in preadipose cells. Mol Pharmacol 1994;46: 1070–1076.

23. Harris PK, Kletzien RF: Localization of a pioglitazone response element in the adipocyte fatty acid-binding protein gene. Mol Pharmacol 1994;45: 439–445.

24. Tontonoz P, Hu E, Graves R, Budavari A, Spiegelman B: mPPARgamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 1994;8:1224–1234.

25. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA: An antidiabetic thiazolidinedione is a high-affinity ligand for peroxisome proliferator-activated receptor γ. J Biol Chem 1995;270:12953–12956.

26. Berger J, Bailey P, Biswas C, Cullinan CA, Doebber TW, Hayes NS, Saperstein R, Smith RG, Leibowitz MD: Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated recep-

tor-γ: binding and activation correlate with anti-diabetic actions in db/db mice. Endocrinology 1996; 137:4189–4195.

27. Willson TM, Cobb JE, Cowan DJ, Wiethe RW, Correa ID, Prakash SR, Beck KD, Moore LB, Kliewer SA, Lehmann JM: The structure–activity relationship between peroxisome proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. J Med Chem 1996;39:665–668.

28. Moller DE, Greene DA: Peroxisome proliferator-activated receptor (PPAR) γ agonists for diabetes. In: Scolnick E., ed. Drug Discovery—Advances in Protein Chemistry. 2001:181–212.

29. Willson TM, Brown PJ, Sternbach DD, Henke BR: The PPARs: from orphan receptors to drug discovery. J Med Chem 2000;43:527–550.

30. Murakami K, Tobe K, Ide T, Mochizuki T, Ohashi M, Akanuma Y, Yazaki Y, Kadowaki T: A novel insulin sensitizer acts as a coligand for peroxisome proliferator-activated receptor-alpha (PPAR-alpha) and PPAR-gamma: effect of PPAR-alpha activation on abnormal lipid metabolism in liver of Zucker fatty rats. Diabetes 1998;47:1841–1847.

31. Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA: Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1997;272:3406–3410.

32. Thorp JM, Waring WS: Modification and distribution of lipids by ethylchlorophenoxyisobutyrate. Nature 1962;194:948–949.

33. Lohray BB, Lohray VB, Bajji AC, Kalchar S, Poondra RR, Padakanti S, Chakrabarti R, Vikramadithyan RK, Misra P, Juluri S, Mamidi NV, Rajagopalan R: (−)3-[4-[2-(Phenoxazin-10-yl)ethoxy]phenyl]-2-ethoxypropanoic acid [(−)DRF 2725]: a dual PPAR agonist with potent antihyperglycemic and lipid-modulating activity. J Med Chem 2001;44:2675–2678.

34. Cronet P, Petersen JF, Folmer R, Blomberg N, Sjoblom K, Karlsson U, Lindstedt E, Bamberg K: Structure of the pparalpha and -gamma ligand binding domain in complex with az 242; ligand selectivity and agonist activation in the ppar family. Structure (Camb) 2001;9:699–706.

35. Berger J, Leibowitz MD, Doebber TW, Elbrecht A, Zhang B, Zhou G, Biswas C, Cullinan CA, Hayes NS, Li Y, Tanen M, Ventre J, Wu WS, Berger GD, Mosley R, Marquis R, Santini C, Sahoo SP, Tolman RL, Smith RG, Moller DE: Novel PPARg and PPARd ligands produce distinct biological effects. J Biol Chem 1999;274:6718–6725.

36. Leibowitz MD, Fievet C, Hennuyer N, Peinado-Onsurbe J, Duez H, Berger J, Santini C, Marquis RW, Tolman RL, Smith RG, Moller DE, Auwerx J: Activation of PPARd alters lipid metaboism in db/db mice. FEBS Lett 2000;473:333–336.

37. Oliver WR, Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, Xu HE, Sternbach DD, Kliewer SA, Hansen BC, Willson TM: A selective

peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc Natl Acad Sci USA 2001;98:5306–5311.

38. Tontonoz P, Hu E, Spiegelman BM: Stimulation of adipogenesis in fibroblasts by PPAR γ2, a lipid-activated transcription factor. Cell 1994;79:1147–1156.

39. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, Koder A, Evans RM: PPAR gamma is required for placental, cardiac, and adipose tissue development. Mol Cell 1999;4:585–595.

40. Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Kadowaki T, et al: PPARgamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol Cell 1999;4:597–609.

41. Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM: PPARgamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol Cell 1999;4:611–617.

42. Tontonoz P, Erding H, Graves RA, Budavari AI, Spiegelman BM: mPPAR$_{gamma}$2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 1994; 8:1224–1234.

43. Tontonoz P, Hu E, Spiegelman BM: Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor γ. Curr Biol 1995;5:571–576.

44. Schoonjans K, Watanabe M, Suzuki H, Mahfoudi A, Krey G, Wahli W, Grimaldi P, Staels B, Yamamoto T, Auwerx J: Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. J Biol Chem 1995;270:19269–19276.

45. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J: PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J 1996;15:5336–5348.

46. Martin G, Schoonjans K, Lefebvre AM, Staels B, Auwerx J: Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. J Biol Chem 1997;272:28210–28217.

47. Sfeir Z, Ibrahimi A, Amri E, Grimaldi P, Abumrad N: Regulation of FAT/CD36 gene expression: further evidence in support of a role of the protein in fatty acid binding/transport. Prostaglandins Leukot Essent Fatty Acids 1997;57:17–21.

48. Kallen CB, Lazar MA: Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3–L1 adipocytes. Proc Natl Acad Sci USA 1996;93:5793–5796.

49. De Vos P, Lefebvre AM, Miller SG, Guerre-Millo M, Wong K, Saladin R, Hamann LG, Staels B, Briggs

MR, Auwerx J: Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. J Clin Invest 1996;98:1004–1009.

50. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.

51. Hofmann C, Lorenz K, Braithwaite SS, Colca JR, Palazuk BJ, Hotamisligil GS, Spiegelman BM: Altered gene expression for tumor necrosis factor-alpha and its receptors during drug and dietary modulation of insulin resistance. Endocrinology 1994; 134:264–270.

52. Peraldi P, Xu M, Spiegelman BM: Thiazolidinediones block tumor necrosis factor–alpha–induced inhibition of insulin signaling. J Clin Invest 1997; 100:1863–1869.

53. Rebuffe-Scrive M, Krotkiewski M, Elfverson J, Bjorntorp P: Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. J Clin Endocrinol Metab 1988;67:1122–1128.

54. Mayo-Smith W, Hayes CW, Biller BM, Klibanski A, Rosenthal H, Rosenthal DI: Body fat distribution measured with CT: correlations in healthy subjects, patients with anorexia nervosa, and patients with Cushing syndrome. Radiology 1989;170:515–518.

55. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30–kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 2001;98:2005–2010.

56. Berg AH, Combatsiaris TC, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7: 947–953.

57. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–1599.

58. Combs TP, Wagner JA, Berger J, Doebber TW, Wang W-J, Zhang BB, Tanen M, Berg AH, O 'Rahilly S, Savage DB, Chatterjee K, Weiss S, Larson PJ, Gottesdiener KM, Gertz BJ, Charron MJ, Scherer PE, Moller DE: Induction of Acrp30 levels by PPARgamma agonists: a potential mechanism of insulin sensitization. Endocrinology 2002;143:998–1007.

59. Chao L, Marcus-Samuels B, Mason MM, Moitra J, Vinson C, Arioglu E, Gavrilova O, Reitman M: Adipose tissue is required for the antidiabetic, but not the hypolipidemic, effect of thiazolidinediones. J Clin Invest 2000;106:1221–1228.

60. Zierath JR, Ryder JW, Doebber T, Woods J, Wu M, Ventre J, Li Z, McCrary C, Berger J, Zhang B, Moller DE: Role of skeletal muscle in thiazolidinedione

insulin sensitizer action. Endocrinology 1998;139: 5034–5041.

61. Berger J, Biswas C, Hayes N, Ventre J, Wu M, Doebber TW: An antidiabetic thiazolidinedione potentiates insulin stimulation of glycogen synthase in rat adipose tissue. Endocrinology 1996;137:1984–1990.

62. Oakes ND, Thalen PG, Jacinto SM, Ljung B: Thiazolidinediones increase plasma-adipose tissue FFA exchange capacity and enhance insulin-mediated control of systemic FFA availability. Diabetes 2001;50: 1158–1165.

63. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR,]Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. Nature 2001;409:307–312.

64. Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N: Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. J Biol Chem 1998;273:16710–16714.

65. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W: Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 1992;68:879-887.

66. Tugwood JD, Isseman I, Anderson RG, Bundell KR, McPheat WL, Green S: The mouse peroxisome-proliferator-activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. EMBO J 1992;11:433–439.

67. Marcus SL, Miyata KS, Zhang B, Subramani S, Rachubinski RA, Capone JP: Diverse peroxisome proliferator-activated receptors bind to the peroxisome proliferator-responsive elements of the rat hydratase/dehydrogenase and fatty acyl-CoA oxidase genes but differentially induce expression. Proc Natl Acad Sci USA 1993;90:5723–5727.

68. Zhang B, Marcus SL, Miyata KS, Subramani S, Capone JP, Rachubinski RA: Characterization of protein-DNA interactions within the peroxisome proliferator-responsive element of the rat hydratase-dehydrogenase gene. J Biol Chem 1993;268:12939–12945.

69. Plutzky J: Emerging concepts in metabolic abnormalities associated with coronary artery disease. Curr Opin Cardiol 2000;15:416–421.

70. Rubins HB, Robins SJ, Collins D, Iranmanesh A, Wilt TJ, Mann D, Mayo-Smith M, Faas FH, Elam MB, Rutan GH, Anderson JW, Kashyap M, Schectman G: Distribution of lipids in 8,500 men with coronary artery disease. Am J Cardiol 1995;75:1196–1201.

71. Linton MF, Fazio S: Re-emergence of fibrates in the management of dyslipidemia and cardiovascular risk. Curr Atheroscler Rep 2000;2:29–35.

72. Rubins HB, Robins SJ: Conclusions from the VA-HIT study. Am J Cardiol 2000;86:543–544.

73. Staels B, Vu-Dac N, Kosykh V, Saladin R, Fruchart JC, Dallongeville J, Auwerx J: Fibrates down-regulate apolipoprotein C-III expression independent of

induction of peroxisomal acyl co-enzyme A oxidase. J Clin Invest 1995;95:705-712.

74. Marx N, Sukhova GK, Collins T: PPARalpha activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. Circulation 1999;99:3125-3131.

75. Marx N, Schonbeck U, Lazar MA, Libby P, Plutsky J: Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res 1998;83:1097-1103.

76. Kintscher U, Goetze S, Wakino S, Kim S, Nagpal S, Chandraratna R, Graf K, Fleck E, Hsueh WA, Law RE: Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes. Eur J Pharmacol 2000;410:259-270.

77. Pasceri V, Wu HD, Willerson JT, Yeh ETH: Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-γ activators. Circulation 2000;101:235-238.

78. Collins AR, Meeham WP, Kintscher U, Jackson S, Wakino S, Hoh G, Palinski W, Hsueh WA, Law RE: Troglitazone inhibits formation of early atherosclerotic lesions in diabetic and non-diabetic low-density lipoprotein receptor-deficient mice. Arterioscler Thromb Biol 2001;21:365-371.

79. Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK: Peroxisome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. J Clin Invest 2000;106:523-531.

80. Chen Z, Ishibashi S, Perrey S, Osugo J, Gotoda T, Kitamine T, Tamura Y, Okazaki H, Yahagi N, Iizuki Y, Shionoira F, Ohashi K, Harada K, Shimano H, Nagai R, Yamada N: Troglitazone inhibits atherosclerosis in apoliporotein E-knockout mice. Arterioscler Thromb Vasc Biol 2001;21:372-377.

81. Shiomi M, Ito T, Tsukada T, Tsujita Y, Horikoshi H: Combination treatment with troglitazone, an insulin action enhancer, and pravastatin, an inhibitor of HMG-CoA reductase, shows a synergistic effect on atherosclerosis of WHHL rabbits. Athersclerosis 1999;142:345-353.

82. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, Teissier E, Minnich A, Jaye M, Duverger N, Brewer HB, Fruchart J-C, Clavey V, Staels B: PPARα and PPARγ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med 2001;7:53-58.

83. Chawla A, Boisvert WA, Lee CH, Lafitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, Evans RM, Tontonoz P: A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 2001; 7:161-171.

84. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD: A novel therapy for colitis utilizing PPAR-

gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999;104:383-389.

85. Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, Englaro W, Schoonjans K, Derijard B, Desvergne B, Wahli W, Chambon P, Leibowitz MD, Colombel JF, Auwerx J: Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. J Exp Med 2001;193:827-838.

86. Rosenstock J, Raskin P: Hypertension in diabetes mellitus. Cardiol Clin 1988;6:547-560.

87. Simonson DC: Etiology and prevalence of hypertension in diabetic patients. Diabetes Care 1988;11: 821-827.

88. Ghazzi MN, Perez JE, Antonucci TK, Driscoll JH, Huang SM, Faja BW, Whitcomb RW: Cardiac and glycemic benefits of troglitazone treatment in NIDDM. The Troglitazone Study Group. Diabetes 1997;46:433-439.

89. Ogihara T, Rakugi H, Ikegami H, Mikami H, Masuo K: Enhancement of insulin sensitivity by troglitazone lowers blood pressure in diabetic hypertensives. Am J Hypertens 1995;8:316-320.

90. Weston WM, Heise MA, Porter LE, Bakris G, Viberti G, Freed MI: Rosiglitazone-mediated reductions in urinary albumin excretion are associated with changes in ambulatory blood pressure in type 2 diabetes patients. Diabetes 2001;50:A134.

91. Scherbaum W, Goke B: Pioglitazone is superior to acarbose in improving glycemic control in patients with type 2 diabetes. Diabetes 2001;50:A462.

92. Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 1994;331:1188-1193.

93. Tack CJ, Ong MK, Lutterman JA, Smits P: Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance. Effects of troglitazone. Diabetologia 1998;41:569-576.

94. Zhang HY, Reddy SR, Kotchen TA: Antihypertensive effect of pioglitazone is not invariably associated with increased insulin sensitivity. Hypertension 1994;24:106-110.

95. Fonseca VA, Reynolds T, Hemphill D, Randolph C, Wall J, Valiquet TR, Graveline J, Fink LM: Effect of troglitazone on fibrinolysis and activated coagulation in patients with non-insulin-dependent diabetes mellitus. J Diabetes Complications 1998;12: 181-186.

96. Kato K, Satoh H, Endo Y, Yamada D, Midorikawa S, Sato W, Mizuno K, Fujita T, Tsukamoto K, Watanabe T: Thiazolidinediones down-regulate plasminogen activator inhibitor type 1 expression in human vascular endothelial cells: a possible role for PPARgamma in endothelial function. Biochem Biophys Res Commun 1999;258:431-435.

97. Itoh H, Doi K, Tanaka T, Fukunaga Y, Hosoda K, Inoue G, Nishimura H, Yoshimasa Y, Yamori Y, Nakao K: Hypertension and insulin resistance: role of per-

oxisome proliferator-activated receptor gamma. Clin Exp Pharmacol Physiol 1999;26:558–560.

98. Satoh H, Tsukamoto K, Hashimoto Y, Hashimoto N, Togo M, Hara M, Maekawa H, Isoo N, Kimura S, Watanabe T: Thiazolidinediones suppress endothelin-1 secretion from bovine vascular endothelial cells: a new possible role of $PPAR_{gamma}$ on vascular endothelial function. Biochem Biophys Res Commun 1999;254:757–763.

99. Kawasaki J, Hirano K, Nishimura J, Fujishima M, Kanaide H: Mechanisms of vasorelaxation induced by troglitazone, a novel antidiabetic drug, in the porcine coronary artery. Circulation 1998;98:2446–2452.

100. Song J, Walsh MF, Igwe R, Ram JL, Barazi M, Dominguez LJ, Sowers JR: Troglitazone reduces contraction by inhibition of vascular smooth muscle cell Ca^{2+} currents and not endothelial nitric oxide production. Diabetes 1997;46:659–664.

Address reprint requests to:
Joel Berger, Ph.D.
Department of Endocrinology and Metabolism
Merck Research Laboratories
P.O. Box 2000
Rahway, NJ 07065

E-mail: joel_berger@merck.com